TanshinoneIIA exerts protective effects via attenuate FTH1, a biomarker and therapeutic target in head and neck Squamous Cell Carcinoma

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Research Article

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Abstract

Background

TanshinoneIIA (TanIIA) refers to one of the major lipophilic bioactive components of S. miltiorrhiza that exerts multiple pleiotropic effects (e.g., anti-inflammatory effects, antioxidant, and anti-tumor effects). Head and neck squamous cell carcinoma (HNSCC) is recognized as the sixth most common cancer worldwide. As indicated from existing studies, ferroptosis may be responsible for its progression. However, the underlying mechanisms have not been overall clarified. This study aimed to screen a functional therapeutic target in HNSCC to specifically analyze its correlation with HNSCC and whether TanIIA can act on it to exert a therapeutic effect.

Methods

Data originating from GEO series GSE6631 and GSE13398 datasets were analyzed with the limma R package on GEO2R to find differentially expressed genes between healthy people from HNSCC patients. The DEG-related PPI network was built with the STRING database, and then the visualization was conducted by Cytoscape. FTH1 expression and mapping were examined with Human Protein Atlas, and FTH1 protein analysis and Kaplan-Meier analysis were conducted to carry out survival analyses. Immunohistochemistry (IHC) and immunofluorescence (IF) were used to localize FTH1 in HNSCCs. The protein expressions were analyzed with Western-blot method. The cell survival and invasion ability were identified by employing CCK-8 and Transwell methods.

Results

A high FTH1 expression in the HNSCCs sample could indicate poor patient outcome. TanIIA at certain concentrations could significantly attenuate FTH1 expression and reduce HNSCCs survival and invasion.

Conclusions

As revealed from this study, FTH1 could act as a promising therapy target in HNSCC and can be inhibited by TanIIA, which was demonstrated to carry out anticancer activities with less side effect.

Background

Head and neck squamous cell carcinoma (HNSC) refers to one of the most lethal malignancies with over 800000 new-onset per year. Mortality rates are elevated with stage of illness, and rates > 50% have been identified in critically ill patients[1]. HNSCC originates from epithelial cells of upper respiratory tract and esophageal mucosa, whereas HNSCC exhibits high heterogeneity as impacted by the complex anatomy and genetic variations in the incidence area. Over the past few years, the existing research on the

correlation between ferroptosis and tumor turns out to be one of the existing research hotspots. Several studies confirmed that the expression levels of ferroptosis proteins varied notably in HNSC, thereby significantly regulating cell proliferation [2] and epithelial mesenchymal transformation[3] and even affecting cisplatin resistance[4]. However, systematic studies focusing on ferroptosis and HNSC have been continuously lacked thus far, and novel precision therapy targets research remains in its infancy.

Over the past decade, ferroptosis has been considered an iron-dependent form of regulated cell death involving lethal, iron-catalyzed lipid damage. Currently, there have been three extensively used ferroptosis biomarkers, i.e., ferritin heavy chain1(FTH1),transferrin receptor protein1(TFR1) and glutathione peroxidase 4 (GPX4)[5]. The prior work of the authors screened the mentioned three proteins and found that only FTH1 had significant differential expression in HNSC.

FTH1 refers to the main subunit for ferritin's function, which covers a ferroxidase active center responsible for regulating the oxidation and integration of ferric ions. It has been extensively accepted that FTH1-mediated regulatory pathways affect numerous biological processes (e.g., development, oxidative stress[6], cell differentiation[7] and neuronal functions[8]). Scaramuzzino L[9] et al. confirmed that upstream of the transcription start site of ferritin heavy chain contained an antioxidant response element, which could protect cells from oxidative damage, thereby avoiding apoptosis by responding to oxidative reactions. In the research field of squamous cell carcinoma, the study by Yang L[10] indicated that FTH1P3 (long non-protein coding RNA ferritin heavy chain 1 pseudogene 3) was significantly up-regulated in Esophageal squamous cell carcinoma(ESCC)tissues and cells and was critical to the course of tumor formation. As proposed by some researchers, FTH1 level is correlated with survival rate of oral squamous cell carcinoma (OSCC)[11]. According to the combined results, a possible mechanism of FTH1 differentially expressed in HNSCC was proposed. Combined with the bioinformatics analysis, it was suggested that FTH1 might be a vital drug target for regulating HNSC.

Tanshinone IIA (TanIIA) acts as one of the liposoluble monomers of Tanshinones. TanIIA exerts multiple pleiotropic effects (e.g., anti-inflammatory effects, antioxidant and anti-atherogenic effects). Over the past few years, considerable domestic and foreign studies indicated that TanIIA exhibits highly potent antitumor activity by inducing apoptosis and triggering autophagy in tumor cells. As revealed from Ming Li[12] et al., TanIIA inhibited the Akt-c-Myc signaling and facilitated E3 ligase FBW7-mediated c-Myc ubiquitination and degradation, thereby eventually down-regulating HK2 expression at the transcriptional level. Furthermore, TanIIA was proved to exhibit significant therapeutic potential for ferroptosis-sensitive tumor cells. Guan et al.[13] demonstrated that TanIIA facilitated lipid peroxidation and up-regulated Ptgs2 and Chac1 expressions, i.e., two markers of ferroptosis. TanIIA research has long been conducted. In previous study, the authors confirmed its significant inhibition of proliferation, and migration of inflammatory factors in vitro, whereas its effects against HNSC were not investigated. In the present study, it was hypothesized that TanIIA may impact the treatment of HNSC by inhibiting FTH1. To verify this hypothesis, the cell invasion and proliferation abilities of HNSCCs were examined after the treatment with 0.5mg/L TanIIA. Subsequently, Western-blot assays were performed for protein expression. The

experiments here elucidated that TanIIA could effectively inhibit FTH1. On the whole, the results here highlighted FTH1 as a vital target for HNSC. Moreover, by bioinformatics analysis, FTH1 was demonstrated to be significantly affluent on HNSC expression at different clinical stages. Based on protein expression and biological behaviors analysis, at a certain level of concentration, TanIIA was experimentally confirmed to be able to inhibit tumor invasion, reduce HNSCCs survival and directly inhibit FTH1.

Materials And Methods

2.1: Data Download

Human head and neck squamous cell carcinoma and normal tissue microarray data were obtained from the geo database(https://www.ncbi.nlm.nih.gov/geo/), Down loaded GSE6631 and GSE13398 datasets,GSE6631was uploaded by M A Kuriakose[14]et.al, which contains paired HNSCC tumor and normal samples from 22 patients were evaluated for differential gene expression on Affymetrix U95A chips. GSE13398 was uploaded by Christopher R Cabanski[15] et.al, contains 8 HNSCC tumor samples and 8 normal tonsil samples.Human Protein Atlas (http://www.proteinatl as.org) was used to validate expression levels of FTH1.

2.2 DEG analysis:

The R language limma package (version 3.4.2) (22) (http://www.bioconductor.org/packages/

release/bioc/html/limma.html) was used to analyze theDEGs among samples. The adjusted $P<0.05$ and $|\log2\text{fold change}|>1$ were taken as the cut-off values. The DEG-related PPI network was established using the STRING[16] database (https://www.string-db.org/) followed using visualization by Cytoscape software (version 3.8.2)[17](http://manual.cytoscape.org)

2.3 FTH1 gene and HNSCC correlations analysis:

Human Protein Atlas (http://www.proteinatl as.org) was used to validate FTH1 relations with HNSCC and further display corralations between FTH1 and tumor stage. The Kaplan-Meier (K–M) survival curves and log-rank test were generated to evaluate the difference in FTH1 high expression group and Low expression group in total TCGA HNSCC cohort.

2.4 Cell acquisition

This work was approved by Ethics Committee of Shanghai General Hospital. HNSCCs were purchased from procell Life Science & Technology Co., Ltd. The obtained cells were cultured and passaged as primary HSCC cell line, Cells were cultured in DMEM medium composed of 10 % fetal bovine serum and 1 % double antibody (penicillin-streptomycin mixture) in 37°C and 5 % CO2 incubator, the third-generation cells were used in this experiment.

Observation of FTH1 by immunohistochemistry and Immunofluorescence staining

FTH1 expression was visualized using immunohistochemistry (IHC) method. Fadu cells were fixed in 4% paraformaldehyde (Beyotime, Beijing, China) for 15 minutes at 37°C, permeabilized using 0.1% Triton X-100 (Beyotime) for 20 minutes, blocked using 5%bovine serum albumin (BSA; Sigma-Aldrich, St Louis, MO) for 1 hour at 37°C, and immunostained using the following antibodie for 2 hours at 37°C, perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol: FTH1:(ab65080) 1/400. One hundred microliters of reaction enhancer from a 2-step IHC kit (Zhongshanjinqiao, Beijing,China) was added to the samples, which were incubated at 37°C for 20 minutes. The samples were then incubated with HRP labeled broad spectrum secondary antibodies (1:1000) for 30 minutes at 37°C, after which the nuclei were counterstained with diaminobenzidine (1:1000; Beyotime) for 5 to 8 minutes. Washed with tap water, samples were stained with hematoxylin for 20 seconds (Beyotime), then dehydrated with different concentrations of ethanol and clarified using a dimethylbenzene solution. Images were captured with an inverted phase-contrast microscope.

IF assays were conducted to identify FTH1 protein expression location.The pre-treatment procedure was the same as that used for IHC. IF was performed with FTH1:(5 μg/ml.,ab65080) antibody for 12 h in the dark, followed by incubation with 1:1000 DyLight 594 Conjugated, Goat Anti-Human IgG (Abbkine Inc., North Chicago,IL). The nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI). Fluorescence images were captured using a fluorescence microscope (Leica DMi8, Wetzlar, Germany).

2.5 Cell survivals:

Cell survivals were measured by CCK-8 method. TanIIA (0.24 mg/L, 0.5 mg/L, 1.0 mg/L) intervention for 24 h,48h,72h served as the treatment group. Grouped cells were grown in 96-well plates and cell survival was measured after 24 hours following the manufacturer’s protocol. CCK-8 and serum-free medium were mixed at a volume ratio of 1:10 and incubated in a 5 % CO2 at 37 ºC for 1 hour. Absorbance at 450 nm wavelength (OD value) was measured by a microplate reader.

2.6 Tumor invasive ability

BD Matrigel frozen in a - 80°C freezer at 4 degrees overnight for 24 h, after which 300ul serum-free medium was added 60ul Matrigel mix (4°C operation), 100ul each of the upper chamber was added (3 chambers). Put into the 37°C incubator, for 5h, serum-free medium and Matrigel were diluted at 1:5, and 50ul was added to each well, incubated 2h in the 37 ° C. The cells were digested, washed 3 times with serum-free medium, counted, and a cell suspension was made. Matrigel was washed 1 time with serum-free medium, 100ul of cell suspension was added to each well; 500ul of conditioned medium containing 20% FBS was then added to the lower chamber. Incubation in 37 ℃ incubator for 24h. Transwell chambers were removed and washed 2 times with PBS, fixed with 5% glutaraldehyde at 4 ℃. Add crystal violet (0.1%) to stain for 5-10 min, leave at room temperature for 0.5 h, wash with PBS twice, wipe off the upper surface cells with cotton ball, then observe under microscope.

2.7 Western-Blot assay

Total proteins were extracted using One Step Animal Tissue/Cell Active Protein Extraction buffer (RIPA; Thermo Fisher Scientific). The cells were seeded in 6-well plates and incubated overnight in 37℃ and 5 % CO2 incubator. All groups were starved for 48 hours in FBS-free medium to achieve synchronization. TanIIA group was intervened with 0.5mg / L concentration. Cells cultured in drug-free medium represented the control group. Samples were separated by 10 % sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using a Bio-Rad Electrophoresis System. The proteins were transferred to a nitrocellulose membrane gel electrophoresis separation. Blocking with 5 % skim milk for 1 hour, Tris-buffered saline with Tween 20 (TBST) was used to wash the membranes. Overnight incubation of the membranes with primary antibodies: FTH1: (1μg/ml, Abcam, ab65080,21kDa); GAPDH (1:500, Abcam, ab8245,36kDa) was done at 4°C. This followed another incubation with a 1:10000 dilution of HRP labeled secondary antibody (Cell Signaling Technology, Danvers, MA, USA) at room temperature for 1 hour. A Bio-Rad ChemiDoc MP was used to expose the membranes, and analysis was performed using Image Lab software (Bio-Rad).

2.8 Statistical analysis

All experiments were repeated in triplicate and the results were presented as the mean ± standard deviation (SD). Statistical analyses were performed using SPSS version 21.0 (IBM, Armonk, NY, USA) and GraphPad Prism 8 (La Jolla, CA, USA). Statistically significant differences were determined at $P<0.05$.

Results

3.1. FTH1 is amplified in HNSC and act as an independent predictor of adverse prognosis.

Using the NCBI database GSE6631 and GSE13398 chip datasets, differential expression algorithms were employed to filter experimentally achieved data to find genes with significant alterations. On the whole, 2004 differentially expressed genes were found in HNSCC, i.e., 983 up-regulated genes and 1021 down-regulated genes, and the PPI network of these DEGs was further mapped based on the mentioned data **(Fig. 1A, B)**. After GPX4, TFR1 and FTH1 were screened, FTH1 was considered with differential expression meaningfully in HNSC. Subsequently, the FTH1 expression in HNSC samples of TGCA cohort was compared with that in normal tissues, and FTH1 was found to significantly increase. Furthermore, the FTH1 expression was confirmed to be significantly correlated with tumor stage.

To gain more insights into FTH1 expression and functions, the Human Protein Atlas dataset was adopted to explore the landscape of FTH1 in all organs **(Fig. 2)**. Compared with healthy controls, FTH1 was significantly overexpressed in HNSCC **(Fig. 2A).** As indicated from the statistical analyses, the expression of FTH1 was significantly correlated with clinical stages and poor survival of HCC patients **(Fig. 2B, E).** It was proved that FTH1 could be critical to multiple diseases and multiple tissues and organ. The tissue-specificity exhibited by FTH1 is worth exploring as it can provide clues for therapy and diagnosis **(Fig. 2C,**

D). This study proved that the expression of FTH1, an emerging therapeutic target for treating different diseases, could significantly affect overall survival of patients.

3.2. FTH1 protein expression.

IHC and IF were further performed to figure out the localization and FTH1 expression in HNSCC. It is generally considered that FTH1expression localized to the microtubules (**Fig. 3A)**. Our IHC stanning result is fully consistent with this conclusion. The staining demonstrated a punctate pattern, suggesting that FTH1 stored in cytoplasmic granules (**Fig. 3B)**. IF assay was performed for further observation, the results complied with IHC, i.e., fluorescence signals were found in cytoplasm, while no expression was reported in the nucleus (**Fig. 3C)**.

3.3. TanIIA down-regulates FTH1 expression in HNSCC.

FTH1 expression in HNSCC was measured by Western blotting assay (**Fig. 3D)**. Set Para cancer tissue as the control. Compared with the control, FTH1 in HNSCCs was significantly overexpressed ($P<0.05$). 0.25mg/L, 0.5 mg/L and 1mg/L TanIIA were adopted to intervene in the respective group, respectively. This study confirmed that TanIIA at 0.5 mg / L could effectively down-regulate FTH1 expression in HNSCCs. It is noteworthy that P value in Para cancer group showed no significance as compared with that of Para cancer + TanIIA group. Whether such a result indicated selectivity of TanIIA should be investigated in depth.

3.4. TanIIA effectively reduces HNSCCs survival and invasion

Subsequently, CCK-8 method was adopted for cell survival detection (**Fig. 4A-C)**. TanIIA intervention was performed at 0.25mg/L, 0.5mg/L,1.0mg/L. The survival rate of the respective group was examined at 24h, 48h, 72h, respectively. By determining the mean value, the proportion of living cells of the HNSC group (48H: 70.3%;72H: 52.29%) decreased significantly($P<0.05$) as compared with the control (48H: 67.83%;72H: 54.35%). The effect of TanIIA was represented by a certain time dose effect curve (**Fig. 4D)**. To observe the migration and invasion of HNSCCs, Transwell method was applied (**Fig. 4E-F)**. As indicated from the results, HNSCCs exhibited stronger migration and invasion ability than the control, TanIIA intervention significantly reduced the invasion ability of HNSCCs ($P < 0.05$). At 0.5mg/l TanIIA intervention (**Fig. 4G)**, although the invasion ability of HNSCC (mean = 146) remained higher than that of the control (mean = 119), the difference was not statistically significant ($P=0.0557$), thereby demonstrating that TanIIA intervention effectively inhibited the invasion ability exhibited by HNSCCs. Moreover, by comparing 0.5mg/L TanIIA intervention among controls, no statistical significance was reported($P=0.0964$; $P =0.4841$), which indicated that TanIIA could exhibit high effectiveness and exert less side effect.

Discussion

Head and neck cancer is recognized as the sixth most frequent malignant tumor worldwide, and over 90% of these cancers are head and neck squamous cell carcinomas (HNSCC)[18]. At present, the aetiology and pathogenesis of HNSCC are the subjects of ongoing research and debate. The development of HNSCC refers to a multi-step process involving multiple signal transduction pathways and complex cross-talks among the pathways, exhibiting a sophisticated behavior. Recently, the rapid advancement of microarray technology has significantly boosted gene expression profiling, gene sequencing and convenient diagnosis of diseases for its characteristics of high-throughput and rapid detection.

Through pre-IHC screen, ferroptosis was found to be correlated with HNSCC tumor pathogenesis, cancer progression and drug resistance. Ferroptosis [19] is gloriously known as “New land of Cell Death”. It refers to an iron dependent, distinct from apoptosis, necrosis and autophagy, as well as a novel form of programmed cell death with the diverse and complex targets involved (e.g., PTGS2, Nox1, fth1, COX2, GPx4 and ACSl4). Iron and iron derivatives are critical to the functioning of ROS-producing enzymes, thereby demonstrating that iron might act as a trigger or mediator of cell death signaling, particularly in ferroptosis. It is further revealed that iron-associated targets are essential for ferroptosis[20].

FTH1, a core functional protein in ferritin metabolism, has been found to be involved in cell proliferation and apoptosis [21], immune response, as well as regulation of body iron balance [22]. Over the past few years, the role of FTH1 in tumors has been more extensively investigated. As indicated from Muhammad JS[23]et al., FTH1 is highly expressed in primary liver tumors, and a lower expression is correlated with better survival. They further proved that the silencing of FTH1 inhibited carcinogenesis in Hep-G2 cells. By conducting TCGA analysis, Huang H[24]et al. suggested FTH1 expression as an effective prognostic and diagnosis biomarker for renal cell carcinoma (RCC). In this study, the FTH1 expression was analyzed using including GEO database, Gene Expression Omnibus, and Cancer Genome Atlas datasets, while the protein level of FTH1 was analyzed using the Human Protein Atlas database. Furthermore, the correlations between FTH1 and clinicopathologic characteristics and survival time were examined, and the obtained results are consistent with the data of above research.

It is generally accepted that HNSCCs exhibit the biological characteristics of high proliferation and invasion[25], treatment of HNSCC confronts with considerable challenges. In early-stage HNSCC patients, surgery and radiotherapy act as the mainstays of treatment, whereas 70% - 80% of patients are already locally advanced or advanced during the initial presentation. For these patients, the 5-year survival rate was only 40% or even less[26]. As reported from existing studies, the median survival time of patients was only 10 months once HNSCC has recurred or metastasized[27]. Thus, drug therapies are critical to HNSCC treatment. Currently, Cisplatin remains the treatment of choice for HNSCC, whereas its development of resistance and toxicity cannot be ignored. Given all the mentioned, specific therapeutic intervention with fewer side effects should be urgently developed.

Tanshinone IIA refers to a vital lipid soluble monomeric component extracted from Salvia miltiorrhiza Bge. TanIIA has demonstrated potent tumor inhibitory effects against various tumor cells [28], the

underlying mechanism involves regulation of the cell cycle[29], Suppresses cell proliferation and induces apoptosis[30], inhibits tumor invasion and metastasis, inhibits angiogenesis[31], reverses tumor MDR[32] and so on. Hui Xu[33]et al. proved that TanIIA increases the sensitivity to irradiation in laryngeal cancer cells and in vivo laryngeal cancer model. L Ding[34] et al. examined the underlying mechanisms and showed that TanIIA exerted a strong radio sensitizing effect due to an enhanced ROS generation and autophagy. In this study, whether it could repress the HNSCC DEG FTH1 was verified, and CCK-8 and Transwell method were further performed to verify TanIIA effects on HNSCCs survival and invasion ability, as an attempt to examine its pharmacological effects.

As confirmed from the results of this study, a certain concentration of TanIIa (≥ 0.5 mg / L) could effectively inhibit the FTH1 expression, reduce HNCCs survival and inhibit its invasion. Furthermore, by drawing the comparison in the controls, this study indicated that TanIIA exerted less side effect and showed major potential in HNSCC adjunctive therapies area.

This study proposed numerous suggestions, which proved that modulating FTH1 might show therapeutic potentials in HNSC. Based on specific analysis, it was confirmed that TanIIA down-regulated FTH1 and controlled HNSCC survival and invasion abilities. However, this study had several limitations. FTH1 serves as a vital regulator of ferroptosis. FTH1 is generally considered a suppressor of ferroptosis [35]; if so, the underlying relationships of ferroptosis and HNSC are worth investigating. Moreover, since TanIIA is capable of significantly down-regulating FTH1, a question is raised that whether it affects ferroptosis. The correlated cell-signaling pathways should be studied in depth. Most of the common anti-tumor drugs are chemicals, which have some disadvantages (e.g., high price and drug resistance). TanIIA, a natural source of antineoplastic drugs, exhibits indeed multiple advantages for being economic and effective and exerting less side effect, which holds great prospect for a wide range of applications. Besides, more and deeper research will be conducted on TanIIA at vivo, vitro and molecular levels.

Conclusion

This study assessed FTH1 expression in HNSCC in depth. By conducting the bioinformatics analysis, FTH1 was proven to be significantly overexpressed in HNSCC, which adversely affected the tumor stage and prognosis of HNSC. In addition, Western blotting assay was performed to verify the bioinformatics results. Furthermore, through a Chinese medicinal chemical monomer TanIIA intervention, the biobehavioral variations in HNSCC were observed. It was confirmed that TanIIA at a certain concentration effectively down-regulated the overexpression of FTH1 and controlled the cell survival and invasion abilities of tumor cells. By comparing its effects in the control, this study confirmed that TanIIA could act as a promising adjuvant drug with less cytotoxicity.

Abbreviations

TanIIA
tanshinone IIA

HNSC
Head and neck squamous carcinoma
HNSCCs
Head and neck squamous carcinoma cells
DEG
Differential expressed genes
CCK 8
Cell Counting Kit 8
FTH1
Ferritin Heavy Chain
FTH1P3
long non-protein coding RNA ferritin heavy chain 1 pseudogene 3
TFR1
Transferrin receptor protein1
GPX4
glutathione peroxidase 4
ESCC
Esophageal squamous cell carcinoma
DAPI
4',6-Diamidino-2-phenylindole
DMEM
Dulbecco's Modified Eagle's Medium
FBS
Fetal Bovine Serum
IF
Immunofluorescence
IHC
Immunohistochemistry
PBS
Phosphate-Buffered Saline
PI
Propidium Iodide
MDR
Multidrug resistance

Declarations

AUTHORS' CONTRIBUTIONS

WM designed and experiments and drafted the manuscript. WM and JD performed the experiments and analyzed and interpreted the data. LY helped with data collection and analysis. RFH

coordinated the research group and participated in the experimental design. All authors were involved in critically revising the manuscript.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

This work was approved by Ethics Committee of Shanghai General Hospital.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data and materials are stored in the Key Laboratory of ENT of Shanghai General Hospital and can be requested from the first author and corresponding author.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Figures

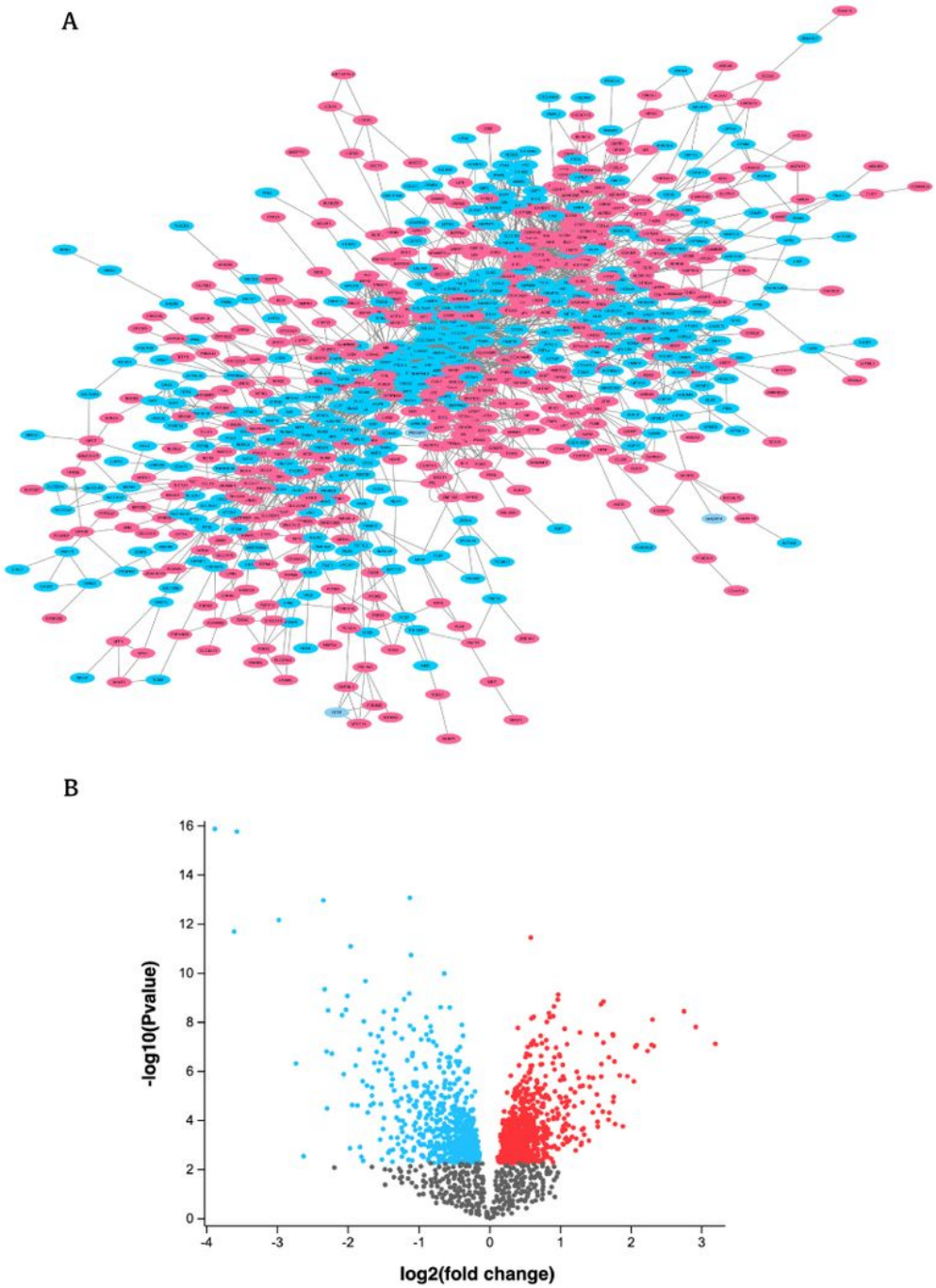

Figure 1

Differentially expressed genes in HNSCC A: PPI network diagrams of differentially expressed genes in HNSCCs. Red represents up-regulated, blue represents down-regulated genes. B Volcano plot of differentially expressed genes in HNSCCs.

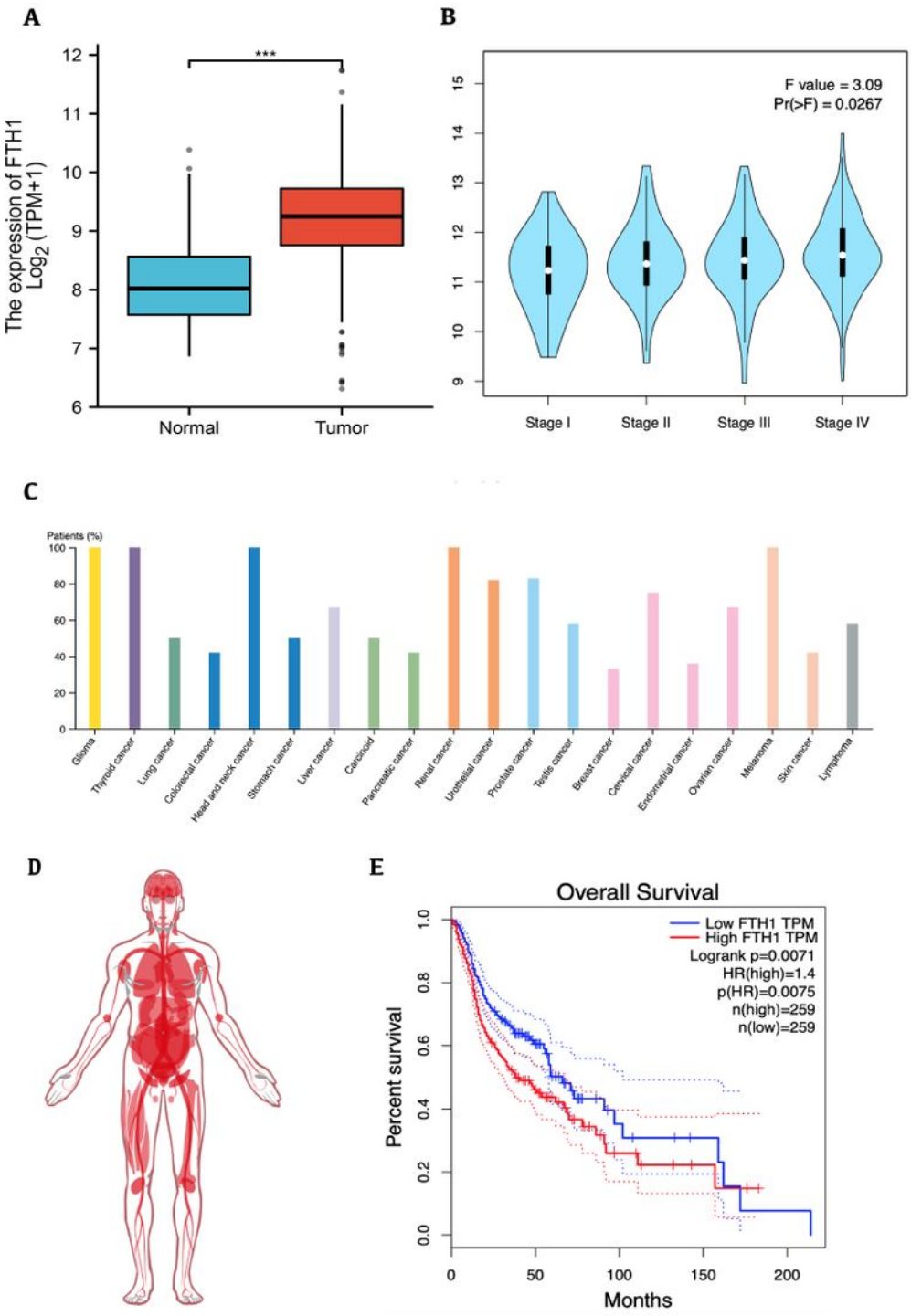

Figure 2

Increased FTH1 expression portend a poor prognosis in HNSCC A: The differentially RNA between healthy controls (n=44) and HNSCC tissues(n=502), data are derived from The Cancer Genome Atlas (TGCA)

database. In tumor samples, FTH1 is significantly overexpressed. B: FTH1 expression correlated with HNSCC clinical stage. C: The Human Protein Atlas database indicate that differential FTH1 expression is prevalent in head and neck cancer patients D: The expression levels of FTH1 in different organs of human. Red represented high level expressions. E: Comparing overall-survival between high FTH1 and low FTH1 patients(n=259). The overall prognosis for high FTH1 is still not optimistic.

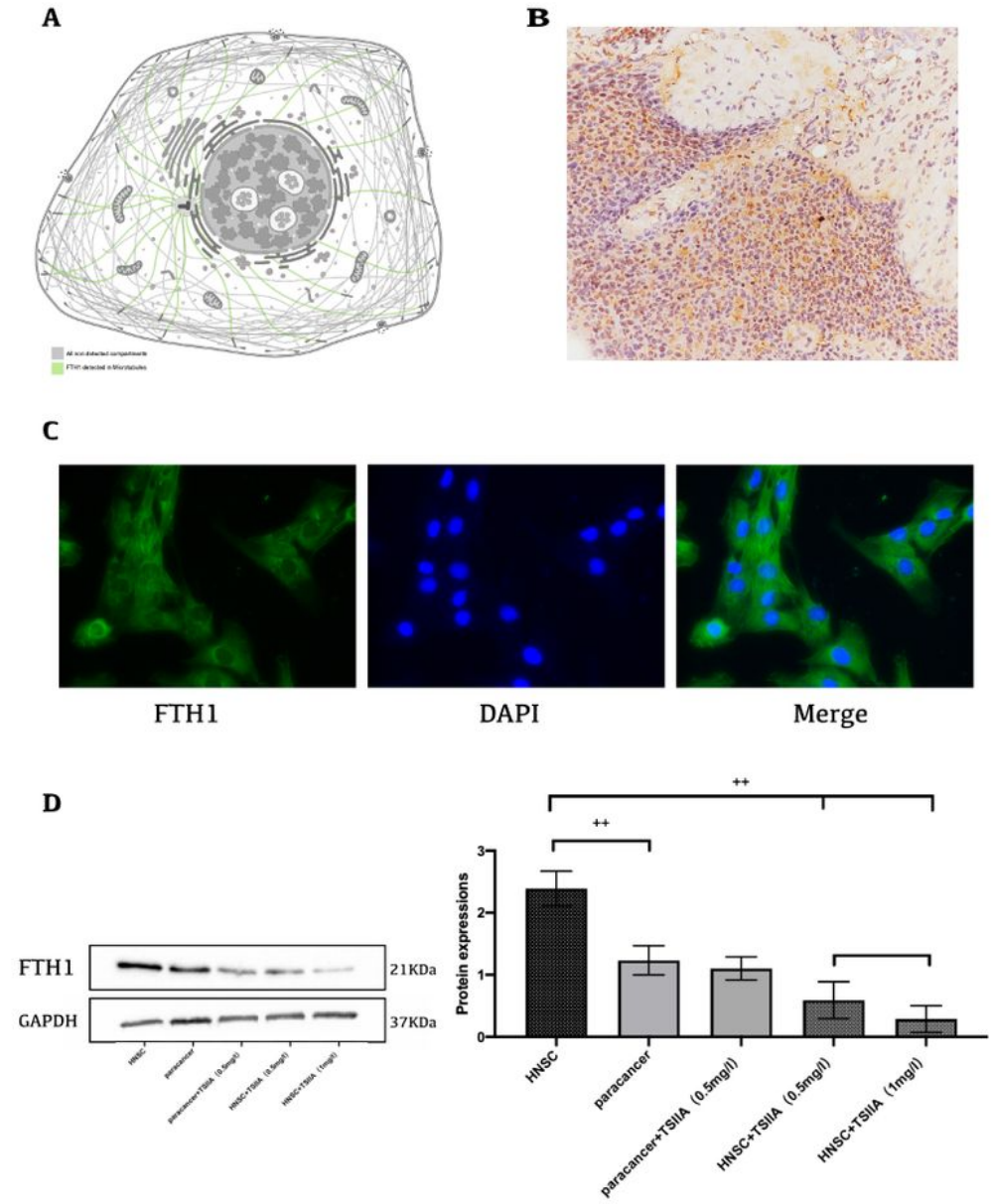

Figure 3

FTH1 expressions in HNSCCs A: FTH1 is expressed in the cytosol and microtubules, with no expression in the nucleus. B: Immunohistochemistry（IHC）staining of FTH1 localized it in the cytoplasm. C: Identification of FTH1 expression by immunofluorescent staining. D: FTH1protein is increased in HNSCCs compared with para cancer controls. TanIIA Intervention （concentration at 0.5mg/L）significantly attenuated FTH1 expression. Data are presented as mean ± SD. +p < 0.05 and ++ p < 0.01 vs the control group.

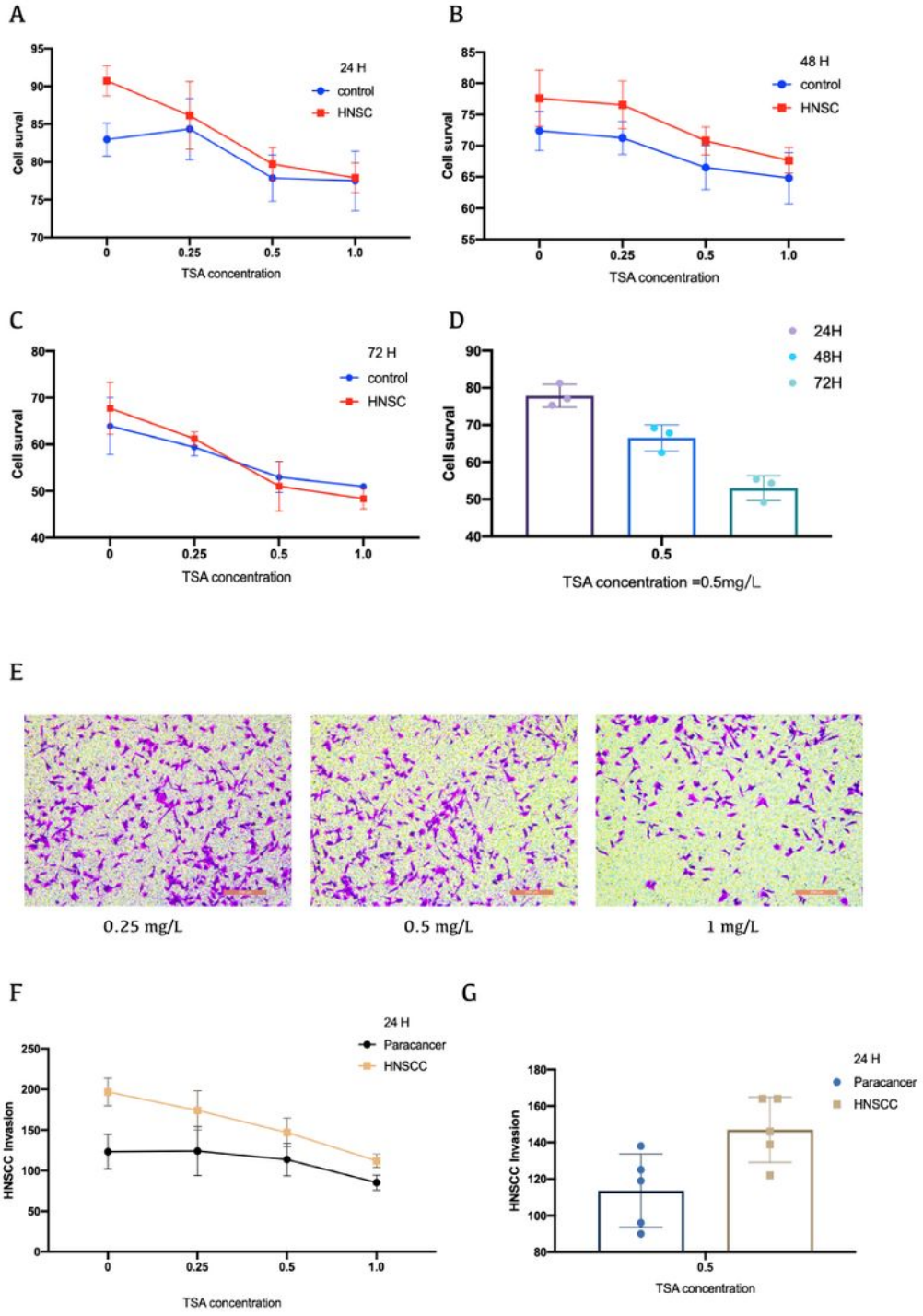

Figure 4

TanIIa effectively inhibited the survival and invasion of HNSCCs A-C CCK-8 kit was used to detect the cell survival rate in 24 hours, 48 hours and 72 hours, respectively. The survival rate of HSCCs after TanIIA intervention was lower than that of the control group with a dose effect relationship. +, $p<0.05$, ++, $p<0.01$, compared with control group. D: Compare each group at a 0.5mg/L concentration TanIIA to assessment whether the effects were enhanced through time. E Invasion ability of HNSCCs under different concentrations (0.25mg/L,0.5mg/L,1mg/L) of TanIIA detected by Transwell assay. F: Compared with control group, HNSCCs have the ability of higher invasion which can be decreased by TanIIA. G: Compare with control group at 0.5mg/L TanIIA at 24h timepoint. P value=0.0557, indicated that TanIIA effectively reduced HNSCCs invasion.

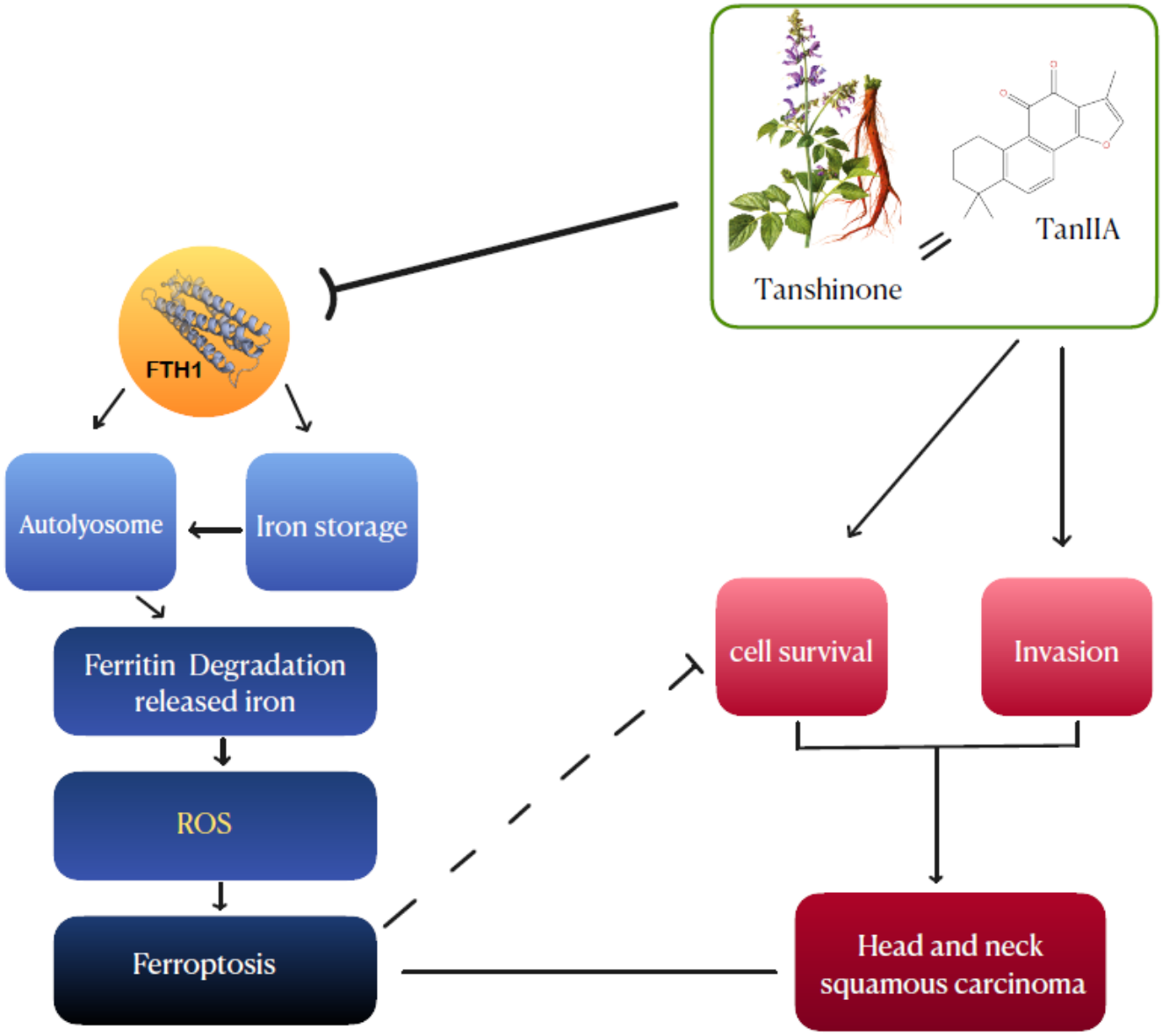

Figure 5

Signal Pathway Map of our study. TanIIA can affect iron metabolism and ferroptosis by attenuate target gene FTH1 also it has a direct inhibitory effect on the survival and invasion of HNSCCs.