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Identification of Cuproptosis-related Gene Lipoyltransferase 1 as a Promising Biomarker in Oral Squamous Cell Carcinoma

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Objective: *To find efficient cuproptosis-related biomarkers to explore the oncogenesis and progression of oral squamous cell carcinoma (OSCC).*

Methods: *All the original data were downloaded from the Cancer Genome Atlas (TCGA) database. Univariate Cox analysis and Kaplan-Meier survival analysis were used to identify the gene related to survival. Tumor Immune Estimation Resource 2.0 (TIMER 2.0) was used to reveal the different expression of cuproptosis-related gene lipoyltransferase 1 (LIPT1) in various kinds of tumours.*

Results: *LIPT1, as a cuproptosis-related gene, was found to be differentially expressed in the OSCC group and the control group. It was also found to be related to the prognosis of OSCC. Pan cancer analysis showed LIPT1 was also involved in various kinds of tumours.*

Conclusion: *All the results demonstrate that the cuproptosis-related gene LIPT1 is highly involved in the oncogenesis and progression of OSCC. These findings give new insight for further research into the cuproptosis-related biomarkers in OSCC.*

Keywords: *biomarker, lipoyltransferase 1, oral squamous cell carcinoma*

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Oral squamous cell carcinoma (OSCC) is a malignant tumour that has been associated with the oral microbiome.[1] Among all oral malignant and premalignant lesions, OSCC has the highest occurrence[2], and the incidence rate increases with age.[3] Despite advancements in treatments, the 5-year survival rate for OSCC remains relatively low at 64%.[4] Thus, there is an urgent need to discover new biomarkers to improve therapy for OSCC.

Recently, immune checkpoint inhibitors targeting programmed cell death protein 1 (PD-1) and programmed death ligand 1 (PD-L1) have been utilised in therapy; however, resistance to immunotherapy is a frequently encountered issue.[5] Therefore, exploring alternative therapies such as ferroptosis and apoptosis may provide new insights into enhancing the survival and prognosis of OSCC patients.[6-8] Cuproptosis, a distinct form of cell death, is triggered by the interaction of copper with lipoylated components of the tricarboxylic acid cycles.[9] However, the specific biomarkers associated with cuproptosis in relation to OSCC remain largely unknown. Thus, the present study aims to explore a novel therapeutic approach for OSCC based on cuproptosis. To achieve this, it is crucial to identify key cuproptosis-related genes, which will further elucidate the underlying mechanisms of OSCC.

The present authors identified 10 cuproptosis-related genes based on the existing literature (Fig 1).[9] Among these genes, lipoyltransferase 1 (LIPT1) exhibited lower expression levels in OSCC samples compared to para-tumour samples. In addition, increased expression of LIPT1 was found to be associated with a poorer

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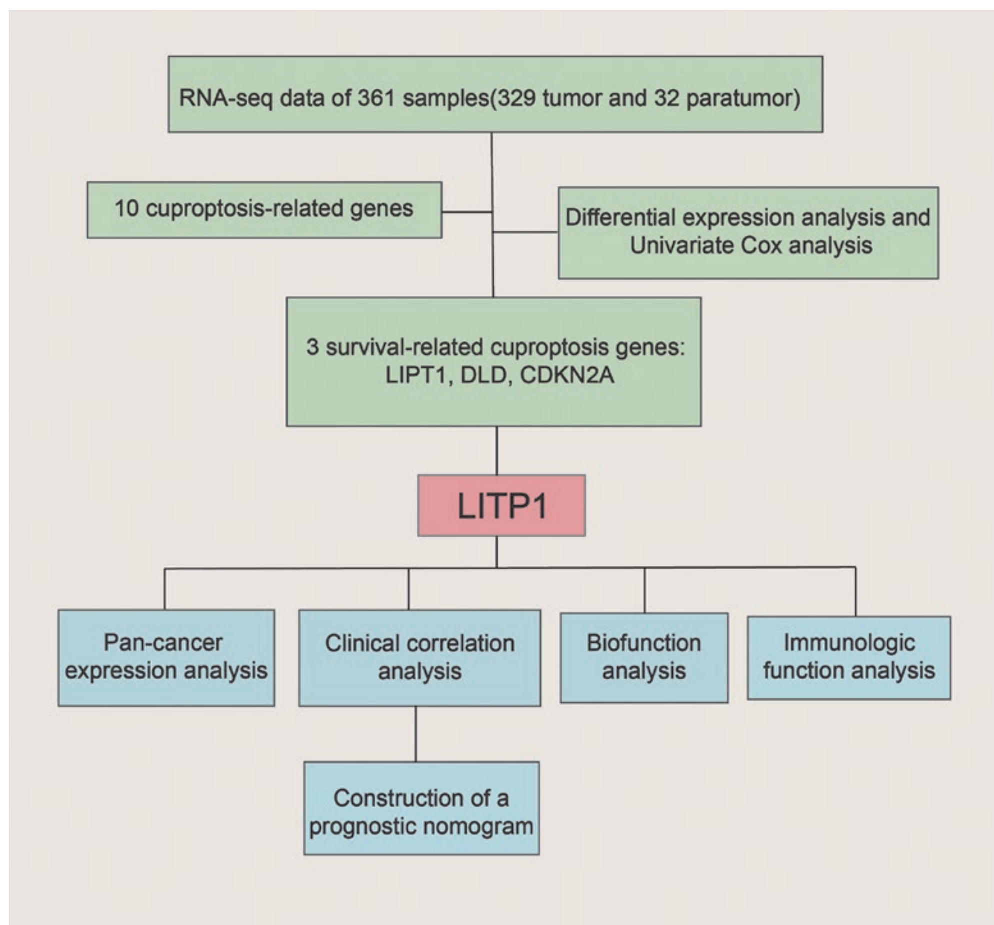

Fig 1 Study flowchart.

prognosis among individuals diagnosed with OSCC. Pan-cancer analysis was conducted to assess the role of LIPT1 across various tumour types. A nomogram incorporating clinicopathological characteristics was developed to predict the 1-, 3- and 5-year overall survival of OSCC patients. To explore the potential mechanisms involving LIPT1, the authors performed an analysis of genes associated with LIPT1. Additionally, Gene Ontology (GO)[10] and Kyoto Encyclopedia of Genes and Genomes (KEGG)[11] enrichment analyses were conducted. The present authors' comprehensive research efforts aim to identify a potential therapeutic target for cuproptosis and provide insights into the prognosis and therapy for OSCC.

Materials and methods

Data acquisition and preprocessing

RNA sequencing data were obtained from The Cancer Genome Atlas (TCGA) and Head and Neck Squamous Cell Carcinoma (HNSC) database (https://portal.gdc.cancer.gov).[12] The data were downloaded and the expression values were converted into Log2 Fragments Per Kilobase Million (FPKM) format. A total of 361 samples with clinical data were selected, excluding samples from non-oral locations such as the hypopharynx, larynx, oropharynx and tonsils. Samples without clinical data were excluded from the analysis.

Identification of cuproptosis-related genes and survival analysis

Ten cuproptosis-related genes, namely ferredoxin 1 (FDX1, Gene ID: 2230), lipoic acid synthetase (LIAS, Gene ID: 11019), LIPT1 (Gene ID: 51601), dihydrolipoamide dehydrogenase (DLD, Gene ID: 1738), dihydrolipoamide S-acetyltransferase (DLAT, Gene ID: 1737), pyruvate dehydrogenase E1 subunit alpha 1 (PDHA1, Gene ID: 5160), pyruvate dehydrogenase E1 subunit beta (PDHB, Gene ID: 5162), glutaminase (GLS, Gene ID: 2744), cyclin dependent kinase inhibitor 2A (CDKN2A, Gene ID: 1029) and metal regulatory transcription factor 1 (MTF1, ID: 4520), were analysed individually to determine the gene that exhibited differential expression between the para-

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tumour group and tumour group samples. Univariate Cox analysis and Kaplan-Meier survival analysis were performed using R survival and survminer software (R Core Team, Vienna, Austria) to identify genes associated with overall survival.[13] The univariate Cox analysis involved fitting a Cox proportional hazards model to the gene expression data using the survival package in R.[14] This model calculates hazard ratios and their associated *P* values, providing a measure of the gene's impact on overall survival. For the Kaplan-Meier survival analysis, the survminer package was used to estimate the probability of survival over time for different gene expression groups. Log-rank tests were performed to compare the survival curves between these groups, determining if there were significant differences in survival outcomes based on gene expression levels. These methods were chosen for their ability to effectively assess the association between gene expression and overall survival in this specific dataset, providing robust statistical analysis for the present study.

Gene expression and clinical prognosis of LIPT1

Expression analysis and clinical characteristics

To assess the expression pattern of LIPT1 across different cancers, the present authors utilised TIMER 2.0 (Tumor Immune Estimation Resource 2.0, http://timer.cistrome.org) to show the expression situation of LIPT1 in different cancers.[15] A Wilcoxon rank-sum test and Dunn test were employed to examine the differential expression of LIPT1 in relation to clinicopathological characteristics such as age, sex and histological grade. These analyses were conducted using the R package ggplot2.[16] Additionally, a nomogram incorporating clinicopathological characteristics was developed using the R packages rms and survival to predict the prognosis of OSCC patients. Furthermore, univariate and multivariate Cox regression analyses were performed to demonstrate that LIPT1 serves as an independent factor for assessing the overall survival of OSCC patients.

Correlation analysis of LIPT1 in OSCC

Pearson correlation analysis was conducted to evaluate the correlation of LIPT1 with other genes, considering a correlation coefficient with an absolute value greater than 0.5 and a significance threshold of $P < 0.05$. The correlation heatmap was generated using the R package ggplot2.

Differential expression analysis and functional enrichment analysis

To identify differentially expressed genes (DEGs), samples were divided into two groups based on the median expression of LIPT1, and DEGs were determined using the criteria $|\text{LogFC}| > 2$ and $P < 0.05$. Functional enrichment analyses, including GO and KEGG analysis, were performed to elucidate the molecular mechanisms and associated signalling pathways of the DEGs. These analyses were conducted using the R package clusterProfiler.[17]

Immune checkpoint and infiltration assay

To explore the relationship between LIPT1 and immune cell infiltration, the present authors utilised the R package GSVA and conducted single-sample Gene Set Enrichment Analysis (ssGSEA). Spearman correlation analysis was conducted to assess the relationship between LIPT1 and immune checkpoint genes, and a correlation heatmap was generated to visualise the results.

Results

Deregulation of LIPT1 in OSCC

In this study, a total of 361 RNA sequencing data samples, consisting of 329 tumour and 32 paratumour samples, were obtained from the TCGA database. Among the ten cuproptosis-related genes analysed, FDX1, LIAS, LIPT1, DLD, DLAT, PDHA1 and PDHB showed higher expression in the paratumour group, whereas GLS and CDKN2A exhibited lower expression in the paratumour group. The gene MTF1 did not show significant differential expression between the tumour and paratumour groups.

Based on univariate Cox analysis (Fig 2a, $P < 0.05$), we identified LIPT1, DLD, and CDKN2A as prognostic genes among the differentially expressed genes. Subsequently, K-M analysis revealed that LIPT1 was specifically associated with overall survival and was selected for further analysis (Fig 2b, $P < 0.05$). Moreover, it has been reported that LIPT1 is involved in various other tumorigenesis, including liver hepatocellular carcinoma, melanoma and prostate cancer.[18-20] The area under the curves (AUCs) for LIPT1 in relation to overall survival was calculated as 0.550, 0.631 and 0.635 at 1, 3 and 5 years, respectively (Fig 2c).[21] Moreover, the authors observed a decrease in the expression level

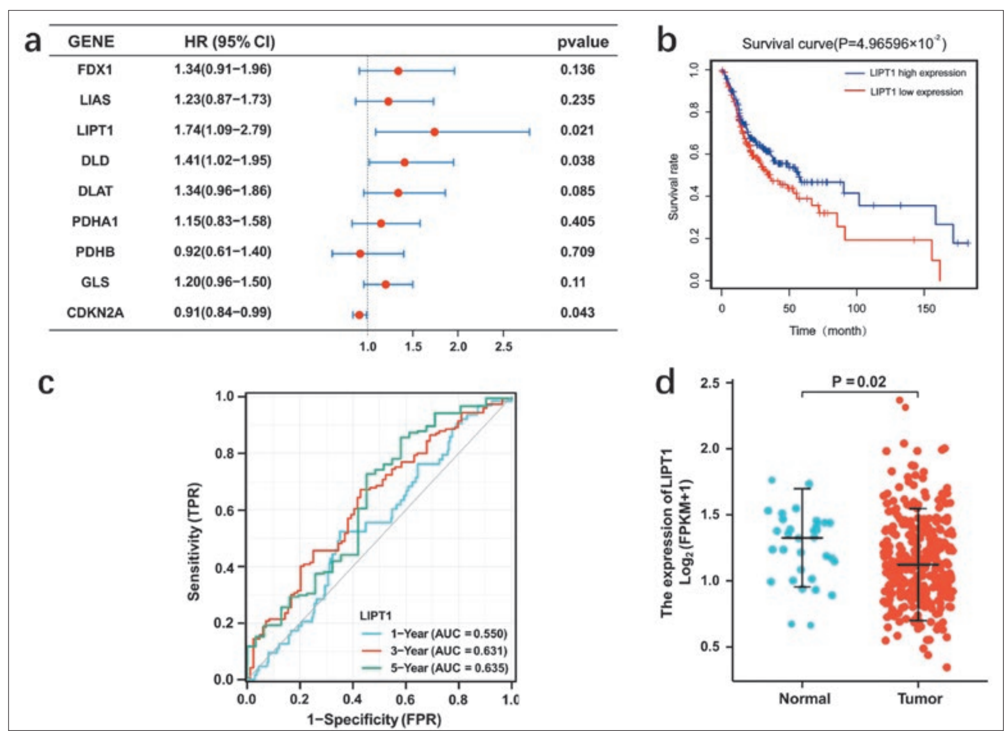

Fig 2 Identification of prognostic genes associated with cuproptosis in OSCC. Univariate Cox regression analysis results presented in a forest plot **(a)**. Kaplan-Meier survival curves illustrating overall survival in OSCC patients based on LIPT1 tumour expression **(b)**. Time-dependent receiver operating characteristic curves showcasing the predictive value of LIPT1 for overall survival at 1, 3 and 5 years **(c)**. Expression of LIPT1 was found to be lower in OSCC samples compared to normal samples **(d)**.

of LIPT1 in the tumour group compared to the paratumour group (Fig 2d).

Pan-cancer expression analysis of LIPT1

A pan-cancer expression analysis of LIPT1 was performed (Fig 3, $P < 0.05$). In CHOL, COAD, ESCA, GBM, LIHC and STAD, LIPT1 demonstrated elevated expression in the tumor group when compared to the normal group. Conversely, in BRCA, CESC, KICH, KIRC, KIRP, THCA and UCEC, LIPT1 exhibited decreased expression in the tumor group compared to the normal group. Additionally, LIPT1 expression was higher in the HNSC-HPV+ group compared to the HNSC-HPV- group, as well as in the SKCM metastasis group compared to the SKCM group.

Differential expression of LIPT1 in clinicopathological characteristics of OSCC and predictive nomogram

Significantly higher expression of LIPT1 was observed in the male group compared to the female group (Fig 4a, $P < 0.05$). In terms of histological grade, LIPT1 exhibited higher expression in grades 3 and 4 compared to grade 1, and higher expression in grades 3 and 4 compared to grade 2 (Fig 4b, $P < 0.05$). A prognostic nomogram was constructed based on clinicopathological characteristics including age, sex, race, smoking history, T stage, alcohol history, histological grade, N stage and LIPT1 expression (Fig 4c). The calibration curves demonstrated that the model fit well with the optimal one, reflecting 1-, 3- and 5-year overall survival (Fig 4d). Table 1 was utilised to conduct univariate and multivariate Cox regression analyses, aiming to determine whether clinicopathological characteristics could act as independent predictors. The results indicated that LIPT1 expression was an independent predictor associated with overall survival.

Genes correlated with LIPT1 and differentially expressed genes in OSCC

Using a Pearson correlation analysis, we identified 300 genes that exhibited a correlation coefficient > 0.5 and a significant P value (< 0.05) with LIPT1 (Table S1). The top 9 genes showing the strongest correlation with LIPT1 were selected to generate a correlation heatmap (Fig 5a). Furthermore, the authors identified 141 genes that showed differential expression between the LIPT1 high expression group and the low expression group, meeting the criteria of |log2FoldChange| > 2 and $P < 0.05$ (data provided on request). Out of these genes, 8 were found to be downregulated whereas 132 were upregulated in the LIPT1 high expression group. GO analysis

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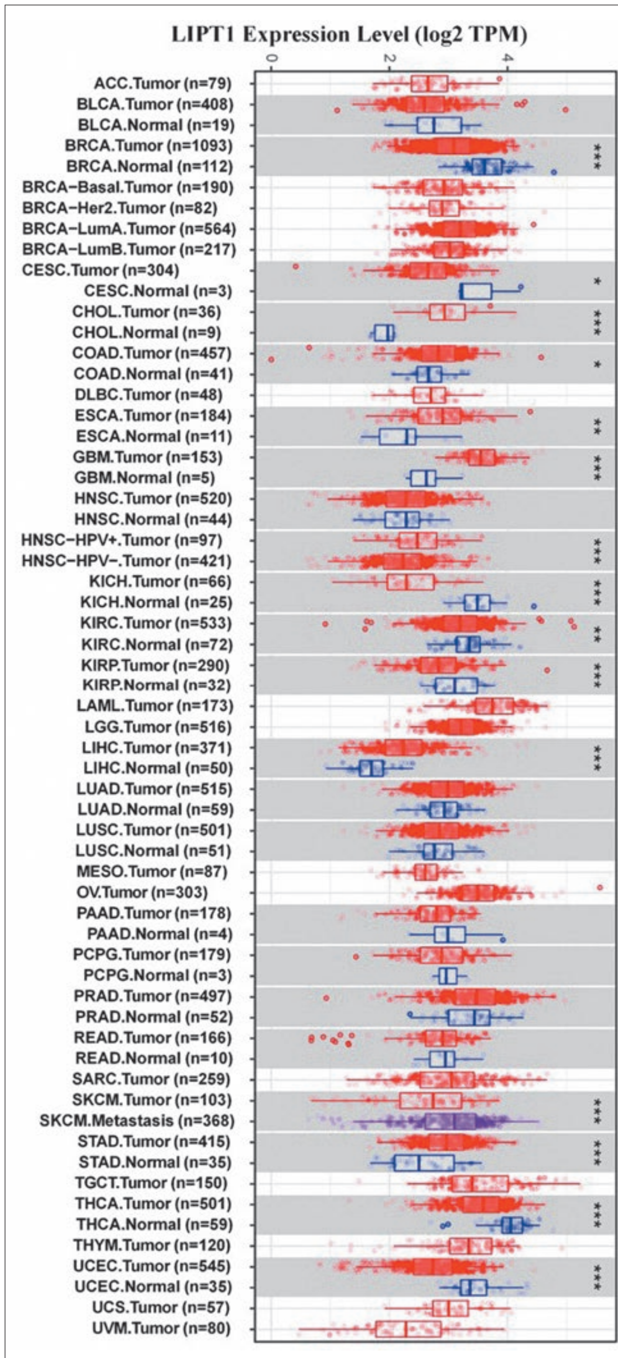

Fig 3 Pan-cancer expression analysis of LIPT1 in normal and tumour samples according to TIMER 2.0. BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; ESCA, oesophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LUSC, lung squamous cell carcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; STAD, stomach adenocarcinoma; THCA, thyroid carcinoma; UCEC, uterine corpus endometrial carcinoma (*P < 0.05, **P < 0.01, ***P < 0.001).

highlighted GO:0001523 (retinoid metabolic process) as the most significantly associated biological process for these genes (Fig 5b). Furthermore, KEGG analysis indicated that the most important pathway involved was hsa00830 (retinol metabolism) (Fig 5c).

Immune cell infiltration and immune checkpoint genes

Using ssGSEA, the relationship between LIPT1 and immune cells was analysed (Fig 6a). It was observed that dendritic cells (DCs), immature DCs (iDCs), mast cells, neutrophils, NK CD56dim cells, central memory T cells (Tcm), Th1 cells, regulatory T cells (Tregs) and eosinophils showed a negative correlation with LIPT1. Conversely, NK CD56bright cells and T helper cells exhibited a positive correlation with LIPT1. Furthermore, the correlation between LIPT1 and immune checkpoint genes was explored, and the results were presented in a correlation heatmap (Fig 6b). LIPT1 showed a positive correlation with TNFRSF25, TNFSF15, TNFSF4, TNFRSF18, TNFRSF4, CD200R1, CTLA4 and TNFRSF14, and a negative correlation with PDCDILG2 (P < 0.05).

Discussion

LIPT1, a pro-cuproptosis gene, is responsible for lipoate transfer and is typically considered downstream of LIPT2.[22] LIPT1 deficiency can manifest as early infantile epileptic encephalopathy, Leigh disease and secondary pyruvate dehydrogenase complex deficiency.[23] LIPT1 also plays a crucial role in the transfer of the lipoyl group from the H-protein of the glycine cleavage system to the E2 subunits of mitochondrial 2-ketoacid dehydrogenase complexes. This process is essential for the proper functioning of these complexes, which are involved in key metabolic pathways such as the tricarboxylic acid (TCA) cycle.[24] Dysfunction of LIPT1 may lead to alterations in TCA cycle metabolism.[25,26] Despite advancements in various treatment modalities, the prognosis for OSCC patients remains poor.[27-29] Cuproptosis has shown potential in overcoming resistance that may arise during chemotherapy treatment of malignant cells; however, the mechanisms underlying cuproptosis, particularly in the context of OSCC, are still largely unknown. Thus, the present authors' research aims to explore potential prognostic biomarkers associated with cuproptosis in OSCC.

A study integrated genomics and metabolomics to identify the cause of lactic acidosis and epilepsy, and revealed that LIPT1 mutations lead to metabolic defects and broader aspects of inborn errors of metabolism

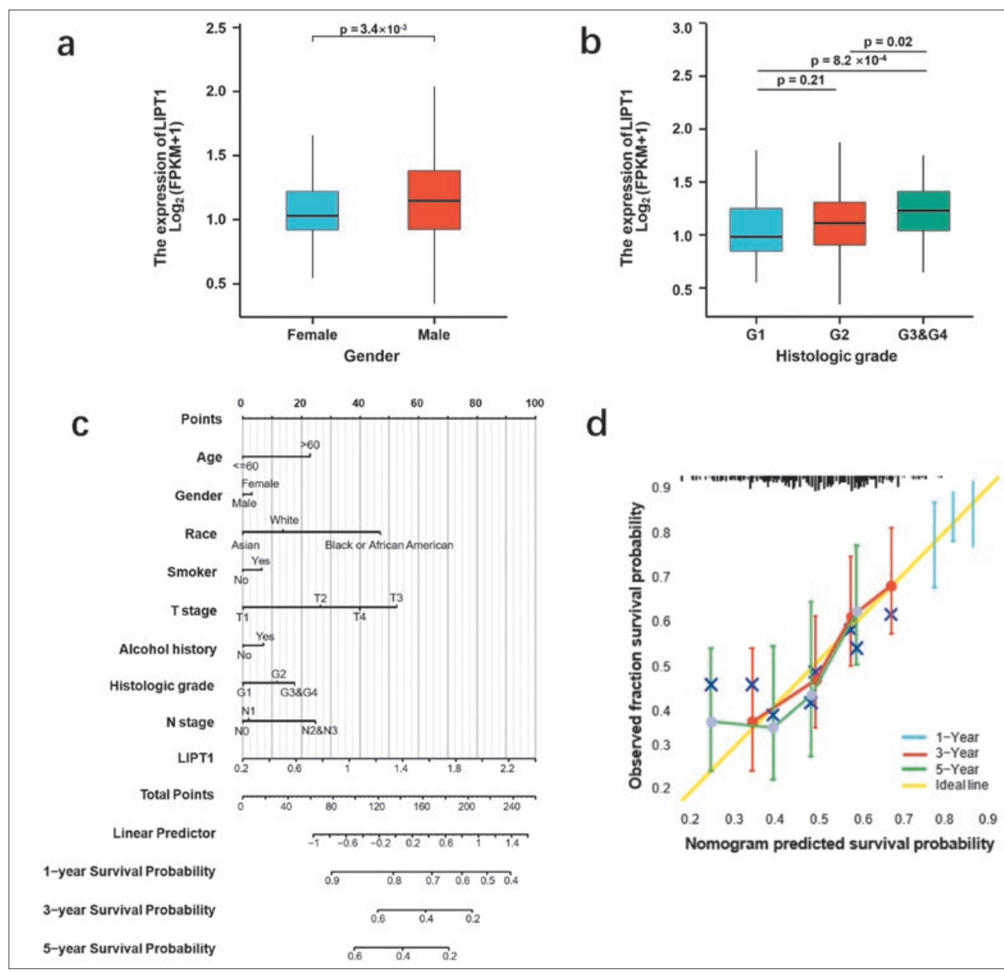

Fig 4 Correlation between LIPT1 and clinical characteristics. Sex distribution in the high and low LIPT1 expression groups **(a)**. Histological grade distribution in the high and low LIPT1 expression groups. G1-4, histologic grade 1-4, based on clinical characteristics **(b)**. Nomogram for predicting overall survival, incorporating age, sex, race, smoking history, T stage, alcohol history, histological grade, N stage and LIPT1 expression as parameters **(c).** Calibration curves of the nomogram for predicting 1-, 3-, 5- and 10-year overall survival **(d)**.

Table 1 Univariate and multivariate Cox regression analysis of clinical characteristics for overall survival prediction.

Characteristics	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Age	1.320 (0.954–1.826)	0.094	1.413 (1.011–1.975)	0.043
Sex	0.908 (0.648–1.273)	0.576	NA	NA
Race	1.109 (0.353–3.485)	0.860	NA	NA
Smoking	1.248 (0.845–1.844)	0.265	NA	NA
T stage	1.289 (0.603–2.755)	0.513	NA	NA
Alcohol history	1.045 (0.740–1.475)	0.804	NA	NA
Histological grade	1.523 (0.950–2.442)	0.081	1.314 (0.798–2.163)	0.283
N stage	1.320 (0.955–1.824)	0.092	1.401 (1.008–1.948)	0.045
LIPT1	1.742 (1.088–2.789)	0.021	1.845 (1.126–3.021)	0.015

NA, not available.

(IEM) pathophysiology.[25] LIPT1 is an essential enzyme for the activation of mitochondrial 2-ketoacid dehydrogenase, which is involved in fatty acylation. LIPT1 deficiency leads to impaired lipoylation and activity of 2-ketoacid dehydrogenase, resulting in increased 2-HG and depletion of structural lipids in plasma. While LIPT1 deficiency impedes lipogenesis, it increases fatty acid oxidation and regulates the balance between oxidative and reductive glutamine metabolism.[25] Pathogenic variants in the LIPT1 gene have been linked to severe lactic acidosis and poor neurocognitive outcomes, leading to neonatal death. A study presented the

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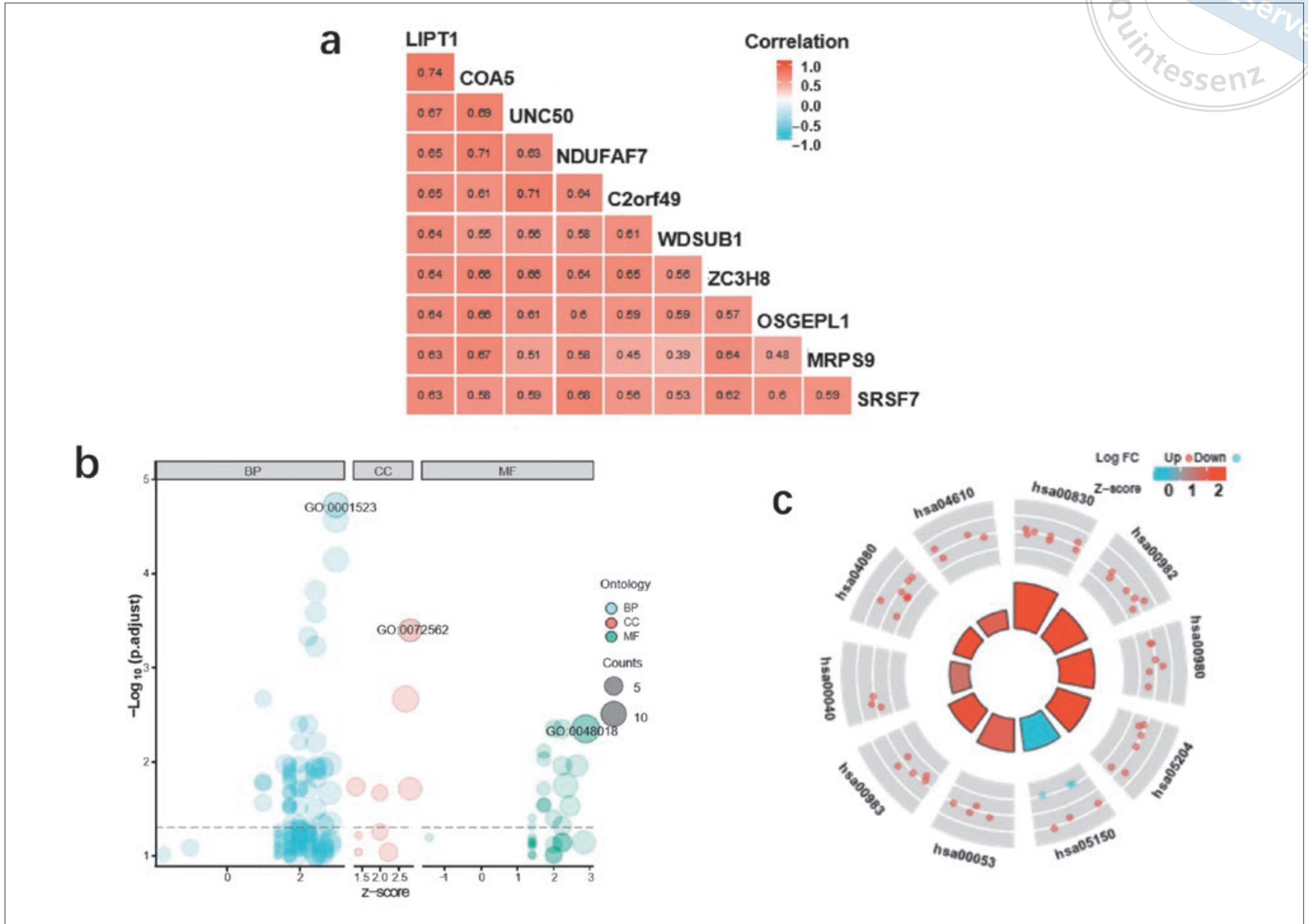

Fig 5 Identification of related genes, pathways and cellular functions of LIPT1. Heatmap showing the correlation of LIPT1 with 9 genes in OSCC samples **(a)**. GO analysis results: GO:0001523 (retinoid metabolic process), GO:0072562 (blood microparticle), GO:0048018 (receptor ligand activity) **(b)**. KEGG enrichment analysis results: hsa00830 (retinol metabolism), hsa00982 (drug metabolism - cytochrome P450), hsa00980 (metabolism of xenobiotics by cytochrome P450), hsa05204 (chemical carcinogenesis), hsa05150 (*Staphylococcus aureus* infection), hsa00053 (ascorbate and aldarate metabolism), hsa00983 (drug metabolism – other enzymes), hsa00040 (pentose and glucuronate interconversions), hsa04080 (neuroactive ligand-receptor interaction), hsa04610 (complement and coagulation cascades) **(c)**.

case of a 2-month-old boy with LIPT1 deficiency and its progression to early infantile epileptic encephalopathy, highlighting the need for exome sequencing to diagnose LIPT1 deficiency and its overlap with other metabolic disorders.[23] LIPT1 has been identified as a novel prognostic target in liver hepatocellular carcinoma and skin cutaneous melanoma.[19,20] In the present study, it was also identified as a prognosis-related cuproptosis gene. The expression of LIPT1 was significantly lower in the OSCC group compared to the paratumour group. However, during the prognostic analysis, the authors discovered that lower expression of LIPT1 correlated with a better prognosis. Cuproptosis may exhibit different effects in distinct situations. On one hand, the upregulation of LIPT1 in the paratumour group suggests that an LIPT1 deficiency might reduce cuproptosis, which plays a role in targeting tumour cell death in OSCC initiation. On the other hand, as the tumour progresses over time, the expression of LIPT1 increases, but the prognosis of OSCC patients worsens. This finding aligns with the observations made by Chen[30] in uterine corpus endometrial carcinoma. Furthermore, in pan-cancer analysis, LIPT1 was found to be downregulated in BRCA, CESC, KICH, KIRC, KIRP and THCA, indicating that LIPT1, as a pro-cuproptosis gene, may contribute to molecular mechanisms underlying cancer initiation and progression. However, the specific mechanisms by which LIPT1 affects the occurrence and progression of OSCC remain unknown.

The findings from the GO and KEGG analyses demonstrated that the DEGs were predominantly linked to metabolic processes, aligning with the observa-

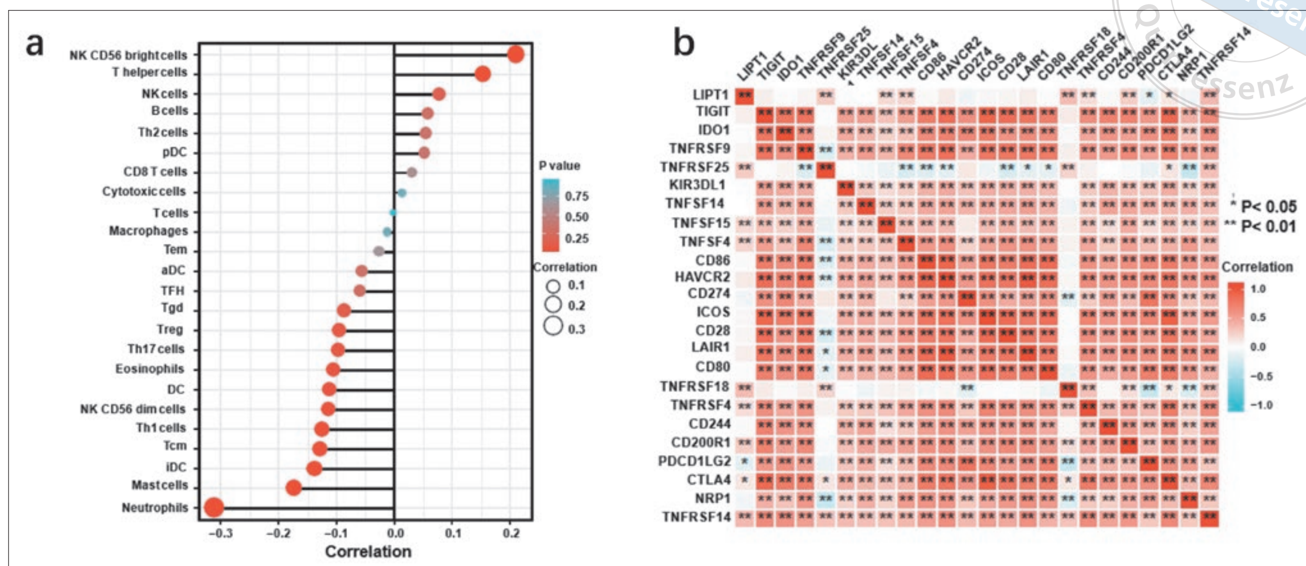

Fig 6 Correlation between LIPT1 and immune infiltration. Lollipop graph depicting the correlation between LIPT1 expression and immune infiltration, as determined by ssGSEA **(a)**. Correlation between LIPT1 and immune checkpoint-related genes **(b)**.

tions made by Zheng et al[31]. Their study revealed that cuproptosis can hinder cancer progression by targeting mitochondrial metabolism.[31] This consistency further supports the present authors' observations. In cancer patients, the tumour microenvironment plays a crucial role in influencing therapy response and clinical outcomes.[32] In the immune cell infiltration analysis, the present authors found a positive correlation between LIPT1 and certain immune cells, including Tregs and neutrophils. Lv et al[19] also reported higher levels of regulatory Tregs and neutrophils in the LIPT1 low expression group in melanoma; however, Jiang et al[33] found a positive correlation between neutrophils and LIPT1 expression in breast cancer. Moreover, mutations in the patient's LIPT1 gene result in a fatal disease characterised by a specific lipoylation defect of the 2-ketoacid dehydrogenase complexes. This patient presented with early onset fatal lactic acidosis and a combined defect of pyruvate dehydrogenase and 2-ketoglutarate dehydrogenase activities, indicating a deficiency in lipoic acid metabolism. Immunostaining analysis revealed reduced lipoylated E2-PDH and E2-KGDH, suggesting a defect in lipoic acid transfer to specific proteins. Sequence analysis identified two heterozygous missense mutations in LIPT1, which were shown to be disease-causing and essential for the activation of 2-ketoacid dehydrogenases in humans.[34]

However, there are certain limitations to consider. Firstly, all the data used in this study were obtained from public databases, and further validation is necessary. Additionally, the 1-year AUC value for overall survival prediction was below 0.6, despite good fit in the calibration curves of the nomogram. Furthermore, DLD and CDKN2A were both found to be associated with overall survival, indicating the potential for developing a prognostic model incorporating these genes and other clinical characteristics in the future.

Conclusion

This study identified LIPT1 as a novel potential molecular biomarker associated with cuproptosis in OSCC.

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Conflicts of interest

The authors declare no conflicts of interest related to this study.

Author contribution

Dr Kuang Min SHEN contributed to the study design, methodology and manuscript draft; Dr Yu Meng ZHOU contributed to the analysis and manuscript draft; Dr Mu Chun LIANG contributed to the data analysis; Dr De Mao ZHANG contributed to the supervision, review

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and editing of the manuscript; Dr Qiang WEI contributed to conception, methodology and supervision; Dr Yi Lin PING contributed to conception, methodology and administration. All authors approved the final version of the manuscript.

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