Original Article - Evaluative Study

# Identification of Novel Cytochrome C1 (CYC1) Gene Expression in Oral Squamous Cell Carcinoma- An Evaluative Study

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## Abstract

**Introduction:** Cytochrome C1 (CYC1) is an important subunit of mitochondrial complex III and plays a vital role in oxidative phosphorylation (OXPHOS) and reactive oxygen species generation. Overexpression of the CYC1 gene has been implicated in cancer development and its prognosis previously, but unexplored in head-and-neck squamous cell carcinomas (HNSCC), especially oral squamous cell carcinoma (OSCC). **Materials and Methods:** CYC1 m-RNA expression and gene alterations were assessed using the Cancer Genome Atlas dataset in HNSCC and validated in OSCC tissues using real-time polymerase chain reaction (RT-PCR). The protein–protein interaction (PPI) network and functional enrichment pathways were also analysed. **Results:** A thorough analysis of the TCGA (The Cancer Genome Atlas) database revealed that CYC1 was overexpressed in the HNSCC cases and the increased expression correlated with several parameters which involve the prediction of advanced diseases such as histopathological grade, tumour-node-metastasis staging, and nodal metastases ($P < 0.05$). The expression of CYC1 was validated using RT-PCR showing significant upregulation ($P < 0.05$) in OSCC tissue samples compared to the normal tissue counterparts. PPI network and functional analysis show the prominent role of CYC1 in OXPHOS, especially in electron transport chain III complex regulation. **Discussion:** The study revealed that CYC1 is highly expressed in HNSCC, and is validated in the OSCC patient tissue samples compared to the normal counterparts and associated with advanced disease stages and grade of the tumour. CYC1 could be a novel promising therapeutic and prognostic marker in HNSCC, especially in OSCC.

**Keywords:** Cytochrome C1, mRNA expression, oral squamous cell carcinoma, prognostic value, the cancer genome atlas database

## Introduction

Head-and-neck squamous cell carcinomas (HNSCC) are the 18th most common type of cancer worldwide.[1] Oral squamous cell carcinoma (OSCC) is the most prevalent type of HNSCC which arises in the oral cavity and accounts for 90% of the HNSCC.[2] OSCC is the most prevalent cancer in Southeast Asian countries such as India and other developing countries like Srilanka.[3] The survival rate of cancers is variable based on various prognostic indicators including grade, stage, metastasis, and invasive ability of the cancer.[4] Owing to the multifactorial pathogenesis of cancer and poor survival rates in advanced diseases, there is a constant quest for identifying novel biomarkers and therapeutic targets. Numerous genes that are implicated in cell cycle progression, apoptosis, tumour suppression, and angiogenesis have been studied extensively to identify a potential theranostic target to overcome the difficulties in the treatment of cancer. Apart from these, alteration in genes implicated in the cellular and mitochondrial energy metabolism also plays a significant role in tumour progression and the overall survival of the malignant cells.[5-7] Consecutively, targeting metabolic or mitochondrial proteins has emerged as a novel technique for treating cancer, particularly metastatic cancers.[8]

In our study, we explored the recent literature and the Cancer Genome Atlas (TCGA) database which is a conglomeration

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of data of m-RNA expression of various genes from patient samples across various cancers including HNSCC. Numerous families of mitochondria-related genes that have been linked to cancer development[9,10] were considered and we identified one novel gene, Cytochrome C1 (CYC1) which has been implicated sparingly in various malignancies.[11,12] CYC1 also known as Ubiquinol-Cytochrome-C Reductase Complex CYC1 Subunit (UQCR4) was first isolated in bovine and yeast cells and assigned to human chromosome 8.[13] The most important function of CYC1 is its integral role in the electron transport chain (ETC) and regulation of the mitochondrial CIII complex.[14] Furthermore, CIII is a dimeric complex which along with CI-CIV forms supramolecular structures, referred to as supercomplexes or "respirasomes".[15,16] It has been suggested that the formation of supercomplexes reduces the amounts of undesired reactive oxygen species (ROS) overproduction.[17] Mutations in CYC1 have been implicated in mitochondrial complex III deficiency, leading to reduced supercomplex formation,[18] causing accumulation of ROS and decreased apoptosis, which are pivotal in cancer development.[15,16]

Although the role of CYC1 has been investigated in various malignancies including breast and osteosarcoma and has also been associated with poor prognosis and resistance to therapy in these tumours,[19,20] the expression of CYC1 and its association with OSCC susceptibility and progression is still debatable. Hence, the study of the expression of such novel biomarkers in early diagnosis and prediction of prognosis is necessary to fill this lacuna.

The present study focussed on assessing the CYC1 gene expression in HNSCC cases using the TCGA dataset and to validate its expression in OSCC tissues and additionally, determine the genetic alterations, the protein–protein interaction (PPI) pathway along with its functional enrichment analysis.

## Materials and Methods

### Patient samples

A total of 28 OSCC patients undergoing surgery were selected at the Department of Oral and Maxillofacial Surgery, Velappanchavadi, Chennai, India, from June 2021 to December 2021 according to the inclusion and exclusion criteria including age, presence or absence of habits, and other systemic diseases. This study was conducted according to the standards obtainable in the 1964 Declaration of Helsinki, as revised in 2013. All the patients were histologically diagnosed cases of OSCC in the department of oral pathology. The study was approved by the Institutional Ethical Committee (IEC), and written informed consent was obtained from all participants (IHEC No. 006/08/2021/IEC/SMCH). Confidentiality was maintained at all times.

### Gene expression analysis of Cytochrome C1 in head-and-neck squamous cell carcinomas using TCGA dataset

The present study initially analysed the CYC1 expression in HNSCC ($n = 520$) and normal tissues ($n = 44$) using patient data from the TCGA dataset (https://portal.gdc.cancer.gov/). Gene expression data along with corresponding clinical data of HNSCC samples were downloaded from the TCGA data portal. University of ALabama at Birmingham CANcer (UALCAN) data analysis Portal (http://ualcan.path.uab.edu/)[21] is a bio-informatics tool which exports results of gene expression and survival analysis from the TCGA database. This was used to analyse the CYC1 expression in primary HNSCC and normal tissues.

### Correlation of clinicopathological parameters of head-and-neck squamous cell carcinomas progression with Cytochrome C1 expression

In addition, the association of clinicopathological parameters with the gene was assessed using the UALCAN analysis tool.[21] Important indicators of disease progression including age and gender, tumour grade, stage, and nodal metastases of the patient were correlated with the gene expression data obtained.[4]

### Validation of Cytochrome C1 expression in oral squamous cell carcinoma patients

The expression of CYC1 was validated in 28 OSCC and 12 adjacent normal tissues using qRT-PCR. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a reference gene and the primers for CYC1 and GAPDH were procured. HYPERLINK "https://paperpile.com/c/hYQ96e/FKSHN"[19] Sequences of primers used for qRT-PCR are CYC1 (Forward 5' -AGCTATCCGTGGTCTCACC-3', Reverse 5' -CCGCATGAACATCTCCCCATC-3') and GAPDH (Forward 5'-GTC GTA TTG GGC GCC TGG TCA CC-3', Reverse 5'-CAC ACC CAT GAC GAA CAT GGG GGC-3').

### RNA isolation, c-DNA conversion, and quantitative real-time polymerase chain reaction

The level of m-RNA expression of CYC1 was determined using qRT-PCR. Total RNA was extracted from the patient tissues using TRIzol Reagent (Invitrogen), and cDNA synthesis kit was used to perform cDNA synthesis according to the instructions of the manufacturer. Then, the purity and concentration of the extracted total RNA were tested using Nanodrop™ (Thermo Scientific™ One Microvolume UV-Vis Spectrophotometer) and the total RNA was subjected to cDNA synthesis. Finally, 1000 ng total RNA was reverse transcribed into cDNA with Oligo (dT) primers in a final volume of 20 µL. The qRT-PCR was performed using the CFX96 Real-Time PCR detection system (Bio-Rad Laboratories Inc., Hercules, CA, USA). Results were expressed at a relative mRNA level and analysed using the comparative threshold cycle (2^ΔCT) method with GAPDH as the reference gene. Statistical analysis was performed using GraphPad Prism 7.0. Student's *t*-test and ANOVA were used to assess the *P* value ($P < 0.05$ was considered statistically significant).

### Protein–protein interaction and functional enrichment analysis

The functional protein association network of CYC1 and their targets was analysed using the STRING (https://string-db.

org/) database.[22] Metascape (http://metascape.org), a web-based portal, was used for a comprehensive functional analysis of CYC1. Further, kyoto-encyclopedia of genes and genomes (KEGG) pathway and process enrichment analysis were performed.

### Genetic alterations of CYC1 assessed using TCGA Database

Assessment of genetic alterations in CYC1, such as amplification, mutations, gain and deep deletions were obtained from TCGA dataset using the cBio Portal tool (cbioportal.org) which is an analysis tool for visualising and exploring large-scale cancer genomic data sets.[23]

## Results

### Cytochrome C1 m-RNA expression is increased in head-and-neck squamous cell carcinomas dataset and oral squamous cell carcinoma patients

Pan-cancer analysis of TCGA data is shown in Figure 1a, which shows the increase in CYC1 expression in numerous cancers, including HNSCC. CYC1 expression in HNSCC was significantly increased compared to the normal tissues ($P < 0.05$) [Figure 1b]. This was validated in our study by comparing the mRNA expression level of CYC1 in OSCC tissues with the adjacent noncancerous tissues [$P < 0.05$, Figure 1c] obtained from patients.

### Cytochrome C1 m-RNA expression is upregulated with advancing grade, stage, and nodal metastases

CYC1 expression correlated with age and gender showing increased mRNA levels in males in the age group of 40–60 years. The expression was correlated with grades based on histopathology and stage of tumour progression based on tumour-node-metastasis (TNM) staging. The nodal metastasis was also associated with the expression levels. CYC1 expression was directly proportional to the increasing grades with the highest expression in poorly differentiated cases ($P < 0.05$) [Figure 2a]. The pathological stage was compared and there was a significant increase in expression between Stage 1 and Stage 4 ($P < 0.05$) [Figure 2b]. Similarly, nodal metastasis of level 3 (N3) showed the maximum expression of CYC1 ($P < 0.05$) [Figure 2c]. The protein expression of CYC1 was visualised in OSCC and normal tissues by immunohistochemistry images [Figure 2d] obtained from the Human Protein Atlas database (https://www.proteinatlas.org).

### Predominant genetic alteration in CYC1 is amplification

The genetic alterations in CYC1 using Oncoprint are depicted in Figure 3a which predominantly showed amplification and minimal association with missense mutation. The copy number status, predominantly amplification and copy gain of CYC1 gene also positively correlated with its mRNA expression in HNSCC patients [Figure 3b]. Copy gain (gain and amplification) of CYC1 was associated with notably increased

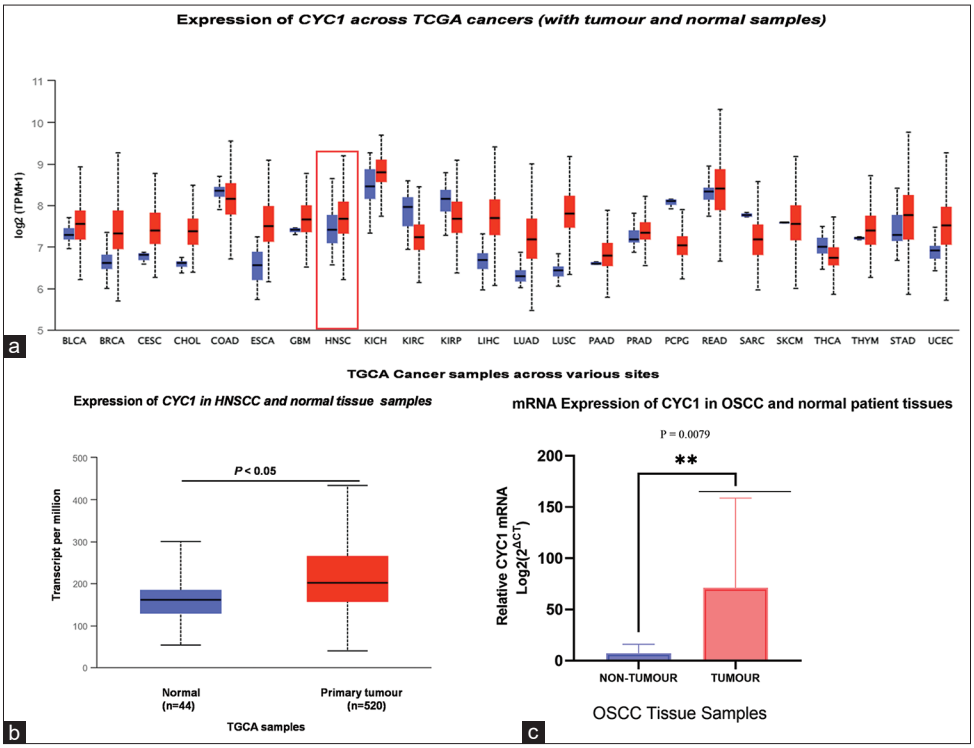


**Figure 1:** mRNA expression and protein expression of CYC1 in HNSCC and normal tissues in TCGA database. Validation of m RNA expression of CYC1 using OSCC tissue samples by RT-PCR. (a) Expression of CYC1 gene across pan-cancer samples in TCGA database compared to normal tissues. (b) Increased CYC1 Expression Pattern in HNSCC compared to normal tissues in TCGA dataset. (c) Highly significant increased expression of CYC1 in OSCC tissue samples compared to normal tissues using RT-PCR ($P = 0.0079$). CYC1 = Cytochrome C1, HNSCC = Head-and-neck squamous cell carcinomas, OSCC = Oral squamous cell carcinoma. RT-PCR = Real-time polymerase chain reaction

**Table 1: Description on the genes, protein encoded, genetic alterations, loci, frequency of variant allele in tumour sample in Cytochrome C1 gene**

| Gene | Protein | Alteration | Copy number variation | Percentage of alteration | Loci | Variant allele frequency in tumour sample | gnomAD frequency data |
|---|---|---|---|---|---|---|---|
| CYC1 | Cytochrome C1 | Gene amplification | | 22 | | | |
| | | H134N | Diploid | | 8 | 0.25 | Novel |
| | | P238L | Gain | | 8 | 0.08 | rs968965290 |
| | | M295I | Diploid | | 8 | 0.35 | Novel |

gnomAD: The Genome Aggregation Database

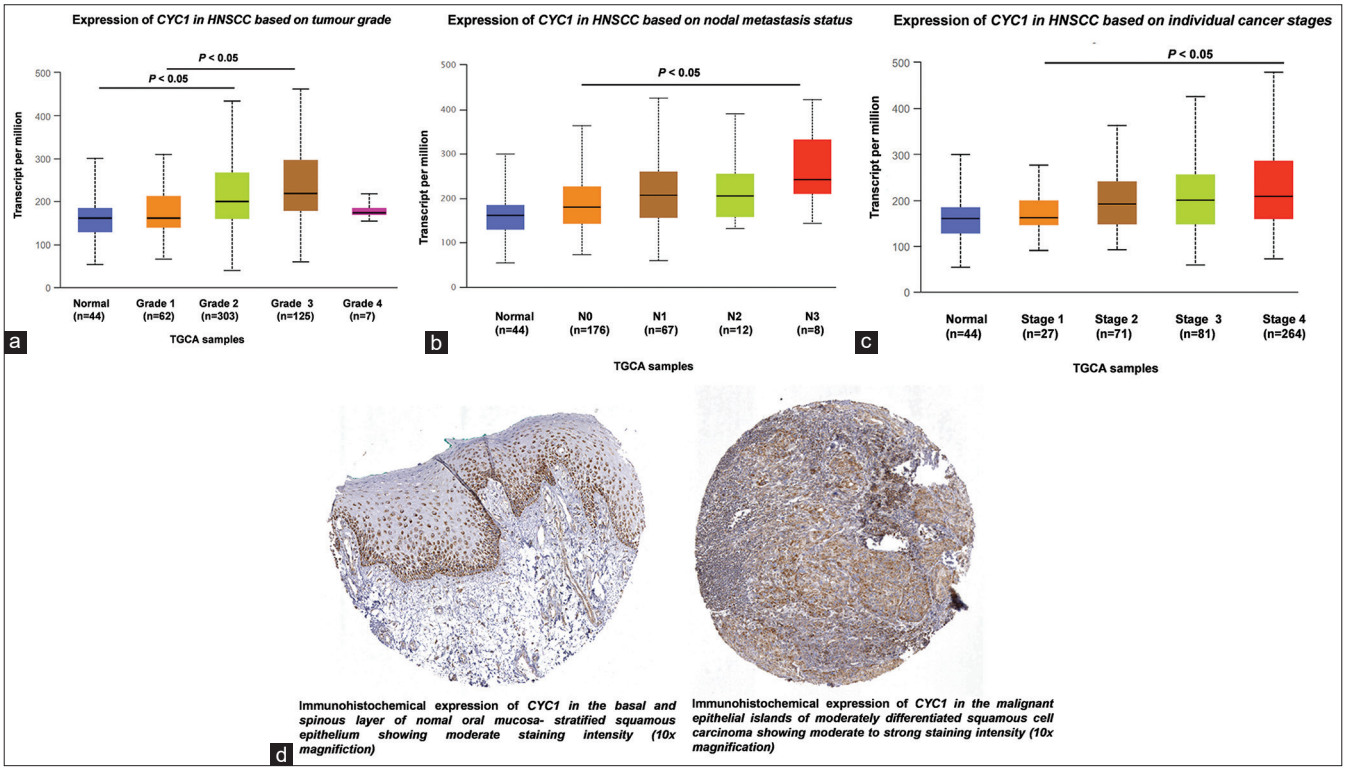


**Figure 2:** Correlation of mRNA expression levels based on histological grading, TNM staging, nodal metastasis along with protein expression data using immunohistochemical images. (a) Association of expression of CYC1 and histological grade in HNSCC tissues and normal tissues with Grade 3 (Poorly differentiated SCC) showing higher expression frequency ($P<0.05$). (b) Association of expression of CYC1 and nodal metastasis status in HNSCC tissues and normal tissues with N 3 (Multiple nodes more than 5 cm) showing higher expression frequency ($P<0.05$). (c) Association of expression of CYC1 and histological grade in HNSCC tissues and normal tissues with Stage 4 (T4b, N1-3, M1) showing higher expression frequency compared to stage 1 ($P<0.05$). (d) Immunohistochemical analysis of CYC1 in normal mucosa (left) and tumour tissue samples (right) with medium positivity in normal and stronger staining intensity in tumour tissue samples (The Human Protein atlas- data).

mRNA levels compared with the copy-neutral (diploid) and copy-loss (shallow deletion) cases. The specific mutations in HNSCC, which are associated with poor prognosis in patients (Stage III and IV disease) are tabulated in Table 1. The schematic representation of the mutations is shown in Figure 3C which shows no novel mutations are associated with CYC1.

## Cytochrome C1 interacts with mitochondrial proteins and modulates mitochondrial electron transport chain and respiratory complex pathways

The PPI network revealed a correlation among various mitochondrial proteins and CYC1 which were identified using the STRING database [Figure 4a]. CYC1 was shown to interact strongly with Cytochrome-C gene (CYCS) and the mitochondrial Cytochrome B gene (MT-CYB) which encodes Cytochrome-C and Cytochrome-B proteins, respectively, as assessed by gene co-expression, text-mining, and protein homology data. The top eight clusters with their representative enriched terms showed that this gene was identified for pathways involved in mitochondrial respiratory chain complex III, mitochondrial electron transport-ubiquinol to Cytochrome C, electron transfer and form an integral part of the membrane, involved in respirasome formation and haeme binding [Figure 4b]. As demonstrated in the KEGG pathway, mitochondrial respiratory chain complex III and mitochondrial electron transport were more enriched, suggesting that these pathways may be closely

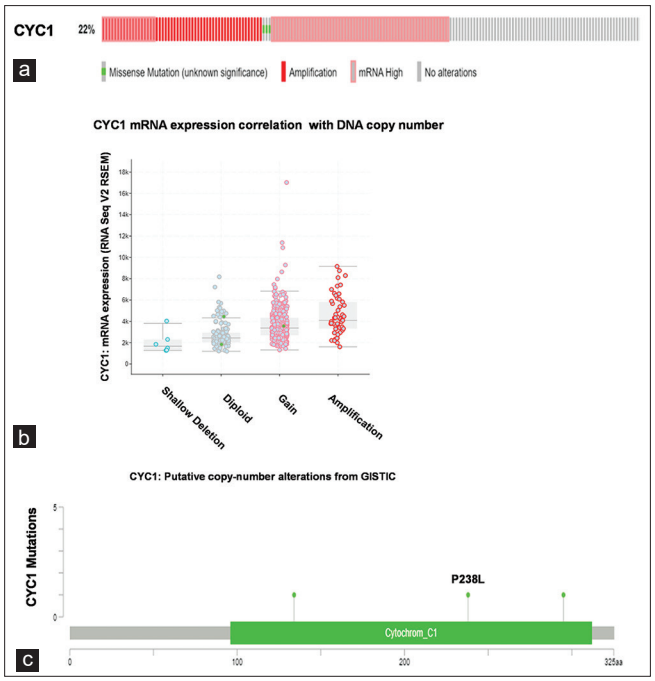


**Figure 3:** Genetic alterations of CYC1 gene with Oncoprint data, copy number variations and mutation associated with the mRNA expression of CYC1 in HNSCC patients. (a) Oncoprint data in cBioPortal database exhibited the proportion and distribution of genetic alterations in CYC1 gene. (b) Copy gain (gain and amplification) of CYC1 was associated with notably increased mRNA levels compared with the copy-neutral (diploid) and copy-loss (shallow deletion) cases. (c) Schematic diagram of CYC1 protein and the positions of mutations.

related to HNSCC development by enhancing ROS formation and oxidative phosphorylation deregulation.

## Discussion

Targeting the mitochondrial proteins appears to be the most recent development in the treatment of cancers. The mitochondrion is a controller of the most important events required for cell survival including a wide variety of pathways for respiration through ETC, cell growth, and apoptosis.[14] It is controlled by a myriad of genes both from within and nucleus. Manipulating these target genes may prove to be crucial in eliminating or limiting the progression of the disease.[12] It is well known that the mechanisms controlling the ETC might be deregulated in cancers depending on the level of dysfunction which can be important therapeutic targets.[24] CYC1 is one such mitochondrial protein which is implicated in controlling mitochondrial metabolism and energy regulation which has been implicated in the progression and metastasis of other malignancies[7,19,20] There is a dearth of information regarding data pertaining to CYC1 expression in HNSCC and OSCC; hence, the present study was designed to assess the expression of CYC1 in OSCC tissues.

In our study, the mRNA expression of CYC1 was preliminarily predicted using the TCGA dataset which consisted of 520 HNSCC cases and 44 normal controls. The expression of CYC1

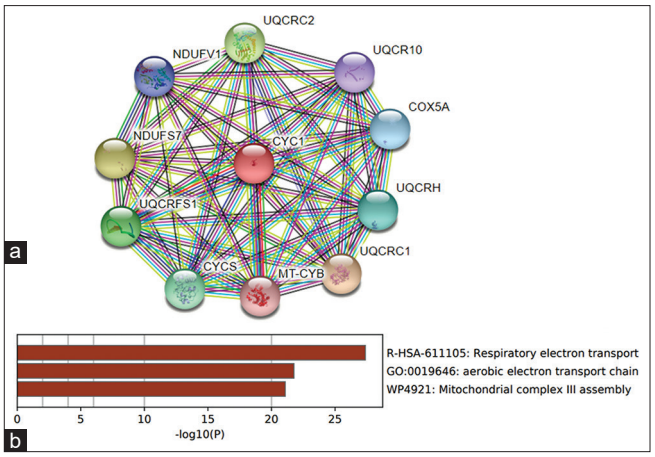


**Figure 4:** Protein-protein interaction network using STRING database and functional enrichment analysis. (a) Protein–protein interaction network between CYC1 and its targets in the STRING dataset. (b) Top 3 functional enrichment pathway analysis heatmap. CYC1 = Cytochrome C1

was found to be significantly increased in HNSCC and also associated with advanced stage, grade, and nodal metastases. Higher grade and stage are prognostic indicators of disease progression. Increased CYC1 expression was associated with poorly differentiated carcinoma compared to well and moderately differentiated carcinomas which show that CYC1 may influence the biological behavior of the tumour, although, grading as a lone parameter is of low prognostic value.[25,26] Higher expression of CYC1 was also noted in Stages 3 and 4 which are often associated with poor prognosis and decreased survival rate of the patient. According to Almangush *et al*., staging and grading form the cornerstone of cancer therapy.[27] Another important criterion which signifies the aggressive and invading nature of the tumour is nodal metastasis. A significant relation has been established between the grade of histological diagnosis and metastasis in the cervical lymph nodes suggesting that an advanced tumour (Grade 3 or 4) has more propensity to metastasise.[28] The CYC1 expression was significantly higher in N3 (which may involve the contralateral nodes) which requires an aggressive treatment protocol to prevent the spread of the tumour.[28]

This concomitant increase in the gene expression with these parameters shows that CYC1 could be related to the progression of the tumour. Similar results with high expression of CYC1 were obtained in a previous study in breast cancer tissues, especially in those which involved lymph node metastasis and was implicated in poor prognosis.[19] Furthermore, in this study, a significant upregulation of mRNA expression in the OSCC tissue samples using RT-PCR, compared to normal tissues, highlighted the possible role of CYC1 in OSCC similar to previous studies.[29] Given the fact that CYC1 overexpression is implicated in tumour progression in previous studies with similar findings, CYC1 may represent a novel target for cancer treatment and prevention.[30]

Furthermore, the genetic alterations assessed using the TCGA database showed association with missense mutations but none

of them was novel according to our results. Missense mutations of CYC1 have been reported earlier in hyperglycaemia and ketoacidosis, apart from cancer.[31] In our study, since the mutations were not novel, it could be considered for any further analysis.

Furthermore, PPI and its functional enrichment analysis showed the crucial role of CYC1 in various mitochondrial functions. Numerous studies have demonstrated that the mechanisms controlling the ETC might be deregulated in cancers depending on the level of dysfunction which can be pertinent therapeutic targets.[24] Our study also reiterates that CYC1 could be an important protein target in OSCC owing to its regulatory role in mitochondrial metabolism.[32]

According to the literature and our observation, although it is clear that CYC1 is required for the adequate functioning of mitochondrial complex III, inhibiting CYC1 completely may also prove to be harmful to normal cells, making it a debatable molecule for therapeutic use.[24] But as a cancer therapy target, based on previous findings, CYC1 knockdown has been shown to efficiently block metastasis in cancer cells.[20] More studies on CYC1 gene expression with a higher sample size and functional analysis of the correlation with tumour susceptibility progression in cell lines or animal models are warranted which is a limitation of our study, especially to analyse its relevance as a therapeutic target.

## Conclusion

The CYC1 upregulation of mRNA expression in the HNSCC cases from TCGA dataset and OSCC patient tissues and the former's association with advancing disease markers such as grade, stage, and metastasis has spotlighted the use of CYC1 as a potential biomarker to indicate the progression of OSCC, especially in metastasis. Despite the enormous research, the prediction of prognosis and progression of cancer is still elusive. Hence, there is an urgent need to unearth such novel prognostic parameters that are sufficiently specific and sensitive to optimise therapy and prognosis with patient stratification in the current scenario.

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### Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

### Conflicts of interest

There are no conflicts of interest.

## References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, *et al*. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71:209-49.
2. Vigneswaran N, Williams MD. Epidemiologic trends in head and neck cancer and aids in diagnosis. Oral Maxillofac Surg Clin North Am 2014;26:123-41.
3. Farah CS, Shearston K, Nguyen AP, Kujan, O. Oral Carcinogenesis and Malignant Transformation. In: Brennan P, Aldridge T, Dwivedi R. edoitors. Premalignant Conditions of the Oral Cavity. Head and Neck Cancer Clinics. Singapore: Springer; 2019.
4. Almangush A, Bello IO, Keski-Säntti H, Mäkinen LK, Kauppila JH, Pukkila M, *et al*. Depth of invasion, tumor budding, and worst pattern of invasion: Prognostic indicators in early-stage oral tongue cancer. Head Neck 2014;36:811-8.
5. Hanahan D. Hallmarks of cancer: New dimensions. Cancer Discov 2022;12:31-46.
6. Aparna J, Smiline-Girija AS, Paramasivam A, Vijayashree-Priyadharsini J. Deciphering the genetic alterations in matrix metallo-proteinase gene family and its putative association with head and neck squamous cell carcinoma. Mol Biol Res Commun 2021;10:13-22.
7. Huang YP, Chang NW. PPARα modulates gene expression profiles of mitochondrial energy metabolism in oral tumorigenesis. Biomedicine (Taipei) 2016;6:3.
8. Hockenbery DM. Targeting mitochondria for cancer therapy. Environ Mol Mutagen 2010;51:476-89.
9. Magraner-Pardo L, Gobelli D, de la Fuente MA, Pons T, Simarro M. Systematic analysis of FASTK gene family alterations in cancer. Int J Mol Sci 2021;22:11337.
10. Ramasubramanian A, Paramasivam A, Ramani P. FASTK family of genes linked to cancer. Bioinformation 2022;18:206-13.
11. Chatterjee A, Mambo E, Sidransky D. Mitochondrial DNA mutations in human cancer. Oncogene 2006;25:4663-74.
12. Yuan Y, Ju YS, Kim Y, Li J, Wang Y, Yoon CJ, *et al*. Comprehensive molecular characterization of mitochondrial genomes in human cancers. Nat Genet 2020;52:342-52.
13. Nishikimi M, Suzuki H, Yamaguchi M, Matsukage A, Yoshida MC, Ozawa T. Assignment of the human cytochrome c1 gene to chromosome 8. Biochem Int 1988;16:655-60.
14. Pacheco R, Quezada SA, Kalergis AM, Becker MI, Ferreira J, De Ioannes AE. Allergens of the urushiol family promote mitochondrial dysfunction by inhibiting the electron transport at the level of cytochromes b and chemically modify cytochrome c1. Biol Res 2021;54:35.
15. Acín-Pérez R, Fernández-Silva P, Peleato ML, Pérez-Martos A, Enriquez JA. Respiratory active mitochondrial supercomplexes. Mol Cell 2008;32:529-39.
16. Nesci S, Lenaz G. The mitochondrial energy conversion involves cytochrome c diffusion into the respiratory supercomplexes. Biochim Biophys Acta Bioenerg 2021;1862:148394.
17. Madkour LH. Biological mechanisms of reactive oxygen species (ROS). Reactive Oxygen Species (ROS), Nanoparticles, and Endoplasmic Reticulum (ER) Stress-Induced Cell Death Mechanisms; Elsevier 2020:19-35.
18. Xia D, Esser L, Tang WK, Zhou F, Zhou Y, Yu L, *et al*. Structural analysis of cytochrome bc1 complexes: Implications to the mechanism of function. Biochim Biophys Acta 2013;1827:1278-94.
19. Han Y, Sun S, Zhao M, Zhang Z, Gong S, Gao P, *et al*. CYC1 predicts poor prognosis in patients with breast cancer. Dis Markers 2016;2016:3528064.
20. Li G, Fu D, Liang W, Fan L, Chen K, Shan L, *et al*. CYC1 silencing sensitizes osteosarcoma cells to TRAIL-induced apoptosis. Cell Physiol Biochem 2014;34:2070-80.
21. Chandrashekar DS, Bashel B, Balasubramanya SA, Creighton CJ,

Ponce-Rodriguez I, Chakravarthi BV, *et al*. UALCAN: A portal for facilitating tumor subgroup gene expression and survival analyses. Neoplasia 2017;19:649-58.
22. Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, *et al*. The STRING database in 2017: Quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Res 2017;45:D362-8.
23. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, *et al*. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 2013;6:pl1.
24. Hardie DG. AMP-activated/SNF1 protein kinases: Conserved guardians of cellular energy. Nat Rev Mol Cell Biol 2007;8:774-85.
25. El-Naggar AK, Chan JK, Takata T, Grandis JR, Slootweg PJ. The fourth edition of the head and neck World Health Organization blue book: Editors' perspectives. Hum Pathol 2017;66:10-2.
26. Akhter M, Hossain S, Rahman QB, Molla MR. A study on histological grading of oral squamous cell carcinoma and its co-relationship with regional metastasis. J Oral Maxillofac Pathol 2011;15:168-76.
27. Almangush A, Mäkitie AA, Triantafyllou A, de Bree R, Strojan P, Rinaldo A, *et al*. Staging and grading of oral squamous cell carcinoma: An update. Oral Oncol 2020;107:104799.
28. Okada Y, Mataga I, Katagiri M, Ishii K. An analysis of cervical lymph nodes metastasis in oral squamous cell carcinoma. Relationship between grade of histopathological malignancy and lymph nodes metastasis. Int J Oral Maxillofac Surg 2003;32:284-8.
29. Liu Z, Wang HY, Long J, Fang WY. Construction of a lentivirus interfering vector targeting Cyc1 and its interfering efficiency in nasopharyngeal carcinoma cells. Nan Fang Yi Ke Da Xue Xue Bao 2010;30:2629-32.
30. Lu T, Liu H, You G. Long non-coding RNA C5orf66-AS1 prevents oral squamous cell carcinoma through inhibiting cell growth and metastasis. Int J Mol Med 2018;42:3291-9.
31. Gaignard P, Menezes M, Schiff M, Bayot A, Rak M, Ogier de Baulny H, *et al*. Mutations in CYC1, encoding cytochrome c1 subunit of respiratory chain complex III, cause insulin-responsive hyperglycemia. Am J Hum Genet 2013;93:384-9.
32. Protasoni M, Pérez-Pérez R, Lobo-Jarne T, Harbour ME, Ding S, Peñas A, *et al*. Respiratory supercomplexes act as a platform for complex III-mediated maturation of human mitochondrial complexes I and IV. EMBO J 2020;39:e102817.