GMR
The Original

Exploring the Roles of Mitochondrial-Associated MicroRNAs of Head and Neck Cancer Stem Cells in Drug Resistance and Tumor Recurrence

Xiaoning Zhu[1,2], Sethu Thakachy Subha[3], Voon Hoong Fong[3], Wee Lin Tan[1] and Yoke Kqueen Cheah[1,4]*

[1]Department of Biomedical Science, Universiti Putra Malaysia, Serdang, Selangor, Malaysia
[2]Department of Hepatobiliary, The Affiliated Traditional Chinese Medicine Hospital, Southwest Medical University, Luzhou, China
[3]Department of Otorhinolaryngology, Universiti Putra Malaysia, Serdang, Selangor, Malaysia
[4]UPM-MAKNA Cancer Research Laboratory, Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia

Corresponding author: Yoke Kqueen Cheah,
E-mail: ykcheah@upm.edu.my

Genet. Mol. Res. 23 (2): gmr2331
Received May 28, 2024
Accepted June 25, 2024
Published June 27, 2024
DOI http://dx.doi.org/10.4238/gmr2331

ABSTRACT. Head and neck cancer (HNC) is one of the most common cancers worldwide. The primary clinical issues in HNC are drug resistance and tumor recurrence attributed to the presence of cancer stem cells (CSCs). Dysfunction of mitochondria may prevent CSCs from apoptosis. MicroRNAs have been reported to be a potential target for diagnosis and treatment strategy in many types of cancers, including HNC. However, the roles of mitochondrial-associated miRNAs in head and neck cancer stem cells remain unclear. First, 18 mitochondrial-associated miRNAs of head and neck cancer stem cells were identified from the MitoCarta3.0 database, head and neck squamous cell carcinoma data in the TCGA database, and cancer stem cell surface markers of HNC by bioinformatic analysis. Then, the data mining determined let-7c-5p, the potential mitochondrial-associated miRNA of head and neck cancer stem cells related to drug resistance and tumor recurrence. Next, RT-qPCR verified the low expression of Let-7c-5p

in head and neck cancer stem cells. After that, we further used bioinformatic analysis to predict that let-7c-5p might mainly target KRAS and BCL2L1 to regulate mitophagy and apoptosis pathways for keeping cancer stem cells alive. Hence, the treatment strategy based on let-7c-5p may provide novel solutions for drug resistance and recurrence in HNC.
Key words: MicroRNA, Mitochondrial; Cancer Stem Cell; Drug Resistance; Tumor Recurrence

ABBREVIATIONS
AR: Androgen Receptor; CSCs: Cancer Stem Cells; DEG: Differential Gene Expression; DEM: Differentially Expressed miRNA; EBV: Epstein-Barr Virus; EZH2: Enhancer of zeste 2 polycomb repressive complex 2 subunit; HNC: Head and Neck Cancer; HNSCC: Head and Neck Squamous Cell Carcinoma; HPV: Human Papillomavirus; KM: Kaplan–Meier Analysis; MYC: MYC proto-oncogene miRNAs: MicroRNAs; mRNAs: Messenger RNAs; NPC: Nasal pharyngeal Cancer; ROS: Reactive Oxygen Species; TCGA: The Cancer Genome Atlas

INTRODUCTION

Head and neck cancer (HNC) is the seventh most frequent cancer worldwide, with addresses tumors including the lip, oral cavity, larynx, nasopharynx, oropharynx, and hypopharynx (Sung et al., 2021). More than 90% of head and neck cancer cases are squamous cell carcinomas. Tobacco, alcohol, human papillomavirus (HPV), and Epstein-Barr virus (EBV) have been linked to HNC (Goldenberg et al., 2004; Marur & Forastiere, 2008). The significant problems of patients with HNC include less response to treatment, tumor recurrence, and metastasis, which reduces the patient's overall survival time. In recent decades, system treatment, including chemotherapy, targeted therapy, and immunotherapy, as the primary option for the recurrence and metastasis of HNC has shown great survival benefits (Mody et al., 2021). Unfortunately, the prognosis of patients with recurrence and metastasis in HNC worsens soon once systemic therapy experiences drug resistance.

Cancer stem cells (CSCs) are proposed to play a critical role in drug resistance and tumor recurrence due to characteristics including ongoing self-renewal, differentiation, and evasion of the immune system (Huang et al., 2020). The fundamental explanation for tumor drug resistance is that CSCs could resist the drugs that lead to DNA damage-induced cancer cell apoptosis in several ways, notably through enhanced reactive oxygen species (ROS) scavenging (Lth et al., 2018). Mitochondrial function is critical in cancer stem cell apoptosis by regulating ROS levels (Wx et al., 2016). However, the relationship between CSCs and mitochondria remains unclear.

MicroRNAs (miRNAs) are small, non-coding RNAs that regulate gene expression by complementary binding to the target messenger RNAs(mRNAs) (Ilieva et al., 2022). MiRNAs can play a significant role in chemotherapy-induced apoptosis by regulating the expression of chemotherapeutic target genes in cancer cells (Lopez & Tait, 2015). Recent studies suggest that miRNAs regulate cancer cell apoptosis, drug resistance, and prognosis in HNC via the mitochondrial pathway (Chen F, 2022; Lo et al., 2020). However, the role of mitochondria-associated miRNAs in the apoptosis of head and neck cancer stem cells and the process behind drug resistance and tumor recurrence remains unknown.

MATERIALS AND METHODS

Ethics approval and consent to participate

This study was reviewed and approved by the Medical Research and Ethics Committee, Ministry of Health Malaysia (NMRR ID-22-01803-NVA).

Public Data collection

Tumoral RNA-seq data, miRNA-seq data, and clinical data from head and neck squamous cell carcinoma (HNSCC) patients were downloaded from The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/), as were mRNA expression data from matching normal tissue samples (Date:29/May/2023). The MitoCarta3.0 database obtained a list of 1,136 human genes encoding proteins with solid support for mitochondrial localization. The 11 HNC cancer stem cell surface marker mRNAs were collected from the systematic review (Singh et al., 2021).

Bioinformatics analysis

Using the R package limma, the TCGA database was used to analyze differential gene expression (DEG) and differentially expressed miRNA (DEM) between tumor and normal tissue samples. DEGs and DEMs were distinguished by having log2 fold change > 2 and p-value < 0.05. Kaplan–Meier (KM) survival curves and survival R package were used to compare the prognosis of DEGs and DEMs in high- and low-expression groups, with p-value < 0.05.

The target miRNAs were predicted from mitochondria- and CSC-related DEGs of HNSCC by the miRWalk V2.0 database. The jvenn online tool merged correlated target miRNAs from mitochondria-, CSC-, and survival prognosis-related target miRNAs.

Data Mining Analysis

The COREMINE database (https://coremine.com/medical/) was employed as a text-mining online tool to identify the correlated target miRNAs of HNC in Drug Resistance and Tumor Recurrence and their evidential experiments. The search strategy is as follows: 1. The search keywords include “MicroRNA,” “Drug Resistance, Neoplasm,” and “Neoplasm Recurrence, Local” were used to obtain drug resistance- and recurrence-related miRNAs in neoplasm; 2. We collected a list of drug resistance-related miRNAs and recurrence-related miRNAs in neoplasm, respectively; 3. The jvenn online tool was used to identify the final target miRNAs by merging drug resistance- and recurrence-related miRNAs in neoplasm and Mito-miRNAs of HNCSC. The different expression of let-7c-5p was determined in the ENROCI database (https://rnasysu.com/encori/), and the prognosis efficacy of let-7c-5p was identified in the Kaplan Meier plotter (https://kmplot.com/analysis/).

The Onco.io database (https://onco.io/) was used to explore the interactions of let-7c-5p and its target genes with the biological processes in HNC. Meanwhile, we draw a regulatory network diagram to demonstrate this interaction. The Enrichr database (https://maayanlab.cloud/Enrichr/) was used to investigate the functional pathways of target genes and potential drugs.

Cell Culture and Cancer Stem Cell Culture

Primary cancer tissue and normal paired non-cancerous tissue were collected from a 44-year-old female Nasopharyngeal Cancer (NPC) patient with written informed consents at Hospital

Pengajar Universiti Putra Malaysia (HPUPM) for the primary cell culture. Initially, the tissues are washed twice in the operating room with 1xPBS containing 1% penicillin/streptomycin (PS). Then, they are moved to the laboratory in a culture medium (DMEM with 10% fetal bovine serum (FBS) and 1% PS) within an hour for further processing. Cancer and pared normal tissues are sliced into 1-2mm fragments using Collagenase II in a Petri dish. The fragments are then transferred to a tube containing 10ml Collagenase II solution and agitated at 37 ℃ for 45 minutes. During this time, the tube is shaken (200 RPM shaking) and vortexed every 15 minutes. After 45 minutes, the mixture is passed through a 70μm cell strainer and washed twice with a complete medium. Finally, the collected cells are placed in a new T25 flask with a culture medium and incubated under recommended conditions (5%CO2, 37℃).

The FaDu and CAL-27 cell lines were obtained from the Chinese Academy of Science Type Culture Collection Cell Bank (Beijing, China) and The Chinese National Infrastructure of Cell Line Resource (Beijing, China). They cultured in the culture medium (DMEM with 10% FBS and 1% PS) and followed the published protocol (Cree, 2011).

Cancer stem cells were induced from the FaDu, CAL-27 cell lines, and NPC primary cancer cells, suspending cultured in the serum-free medium containing the DMEM with Epidermal growth factor (20ng/ml), human Fibroblast growth factor (20ng/ml),1% N2 supplement, and 1% PS, following the published protocol (Torre-Healy et al., 2017).

Reverse Transcription-Quantitative Polymerase Chain Reaction Analysis

The total miRNA of cells was extracted and quantified using the MiPure Cell/Tissue miRNA Kit (Vzayme, China). The reverse transcription was performed using miRNA 1st Strand cDNA Synthesis Kit (by tailing A) (Vzayme, China). The Lepgen-96 qPCR machine (Lepgen, China) quantified the expression of let-7c-5p using the SYBR green-based kit (Vzayme, China). The expression let-7c-5p was calculated by a comparative 2-ΔΔct method. The expression of U6 was quantified as an endogenous control (Table 1).

Statistical analysis

Statistical analysis was performed by the R packages using R software v 4.2, Prism v9, COREMINE database, Onco.io database, and Enrichr database online tools. If not specified otherwise, $P<0.05$ was considered statistically significant.

RESULTS

Prediction of Mitochondria-Associated MicroRNAs of Head and Neck Cancer Stem Cells in Drug Resistance and Tumor Recurrence

A flow chart illustrating the prediction and determination process is presented in Figure 1A. In the first stage, R studio firstly identified 306 OS-related DEG and 68 OS-related DEM in HNSCC

Table 1. Primers used for RT-qPCR.

Primer	Primer Sequence (5′–3′)	Temperature
Has-let-7c-5p-q-F	gcc tga ggt agt agg ttg tat ggt t	58.2
U6-q-F1	ctc gct tcg gca gca ca	59.4
U6-q-R1	aac gct tca cga att tgc gt	55.6

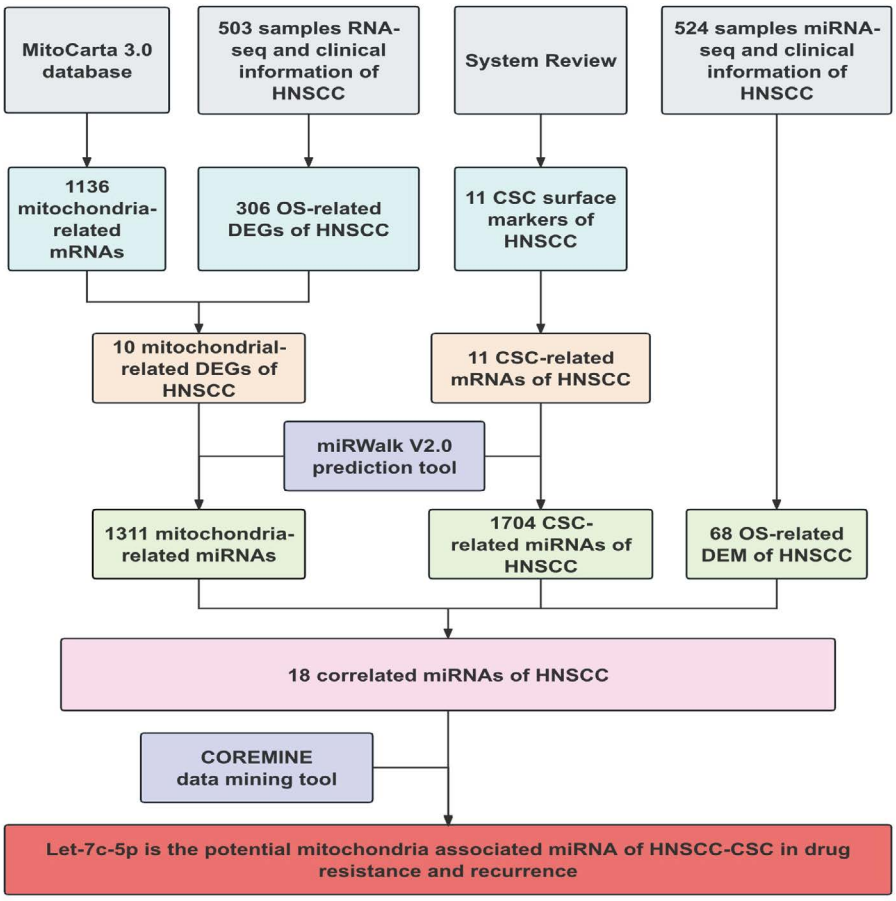

Figure 1. (A) The flow chart of the bioinformatics and data mining analysis process, (B) Venn diagram shows the correlated miRNAs related to mitochondria, CSC, and OS in HNC, (C) Venn diagram shows the mitochondrial-associated miRNAs related to drug resistance and tumor recurrence in head and neck cancer stem cell. OS: overall survival, DEG: differential gene expression, DEM: differentially expressed miRNA.

using 503 (459 tumors vs 44 normal) samples' RNA-seq, 524 (479 tumors vs 45 normal) samples' miRNA-seq, and clinical information from the TCGA-HNSCC database. Then, 1136 mitochondria-related mRNAs and 11 mRNAs of cancer stem cell surface markers in HNSCC were collected from the MitoCarta3.0 database and systematic review. Next, 10 HNSCC mitochondria-related mRNAs were identified by merging 306 OS-related DEG with the 1136 mitochondria-related mRNAs. After that, 1704 and 1311 microRNAs were predicted from 11 mRNAs of cancer stem cell surface markers in HNSCC and 10 HNSCC mitochondria-related mRNAs by miRWalk V2.0. Finally, 18 correlated miRNAs were predicted by merging 1704 mitochondria-related microRNAs, 1311 CSC-related microRNAs, and 68 OS-related DEMs (Figure 1B). In the second and third steps, 101 and 59 miRNAs were evaluated for drug resistance of neoplasm and neoplasm recurrence by the CORMINE database, respectively, and then integrated with 18 mitochondria-associated miRNAs of head and neck cancer stem cells. We determined that hsa-miR-let-7c-5p was involved in these properties (Figure 1C).

Expression and Prognosis Efficacy of Let-7c-5p in HNSCC and HNSCC-CSC

First, the expression of let-7c-5p in HNSCC tissue is lower than in paired normal tissue (FC=0.33, $P<0.05$) (Figure 2A), and the higher expression group of patients showed a better prognosis ($P<0.05$) (Figure 2B). Then, the primary normal cell line from the normal tissue was isolated, and subculture, the classic features of normal epithelial cells were observed (Figure 2C). Next, the FADU-CSC, CAL27-CSC, and primary cancer cells-CSC were induced successfully with the serum-free medium in the low attach condition. The suspended condensed sphere cells with smooth and even peripheries and measured diameters larger than 50 μm were observed under the light microscope (Figure 2D-F). In the end, the RT-qPCR results showed the expression of let-7c-5p was low in the Fadu-, Cal-27-, and primary cancer cell-induced CSC by comparing Normal paired non-cancerous cells ($P<0.01$) (Figure 2G). In addition, the network of let-7c-5p and its target genes interactions is shown in Figure 2H, based on the ONCO database. The green and red lines indicate interaction with positive and negative effects, respectively. The black lines indicate transcriptional regulation, and the black dotted line indicates binding.

Investigation of Biological Processes for Target Genes of let-7c-5p in Cancer

Based on the ONCO database, the 15 genes that let-7c-5p regulates had the most significant effects on regulating biological processes such as migration, invasion, metastasis, tumor growth, cell spreading, apoptosis, cisplatin resistance, and stemness (Table 2). Then, the Enrichr database investigated these target genes' functional pathways and possible drugs. A total of 17 signaling pathways were collected in Table 3 after removing explicitly referring to pathways of other diseases ($P<0.01$). Notably, the pathways related to the cancer stem cell and mitochondria were included, such as Signaling pathways regulating pluripotency of stem cells, Cell cycle, Mitophagy, Central carbon metabolism in cancer. In addition, we identified five potential drugs of the target genes (KRAS and BCL2L1) involved in the mitophagy pathway by Illuminating the Druggable Genome (IDG) database (Table 4).

DISCUSSION

Numerous miRNAs with distinct functional categories have formed intricately regulated networks in tumor genesis and development that interact with multiple mRNAs due to the growing

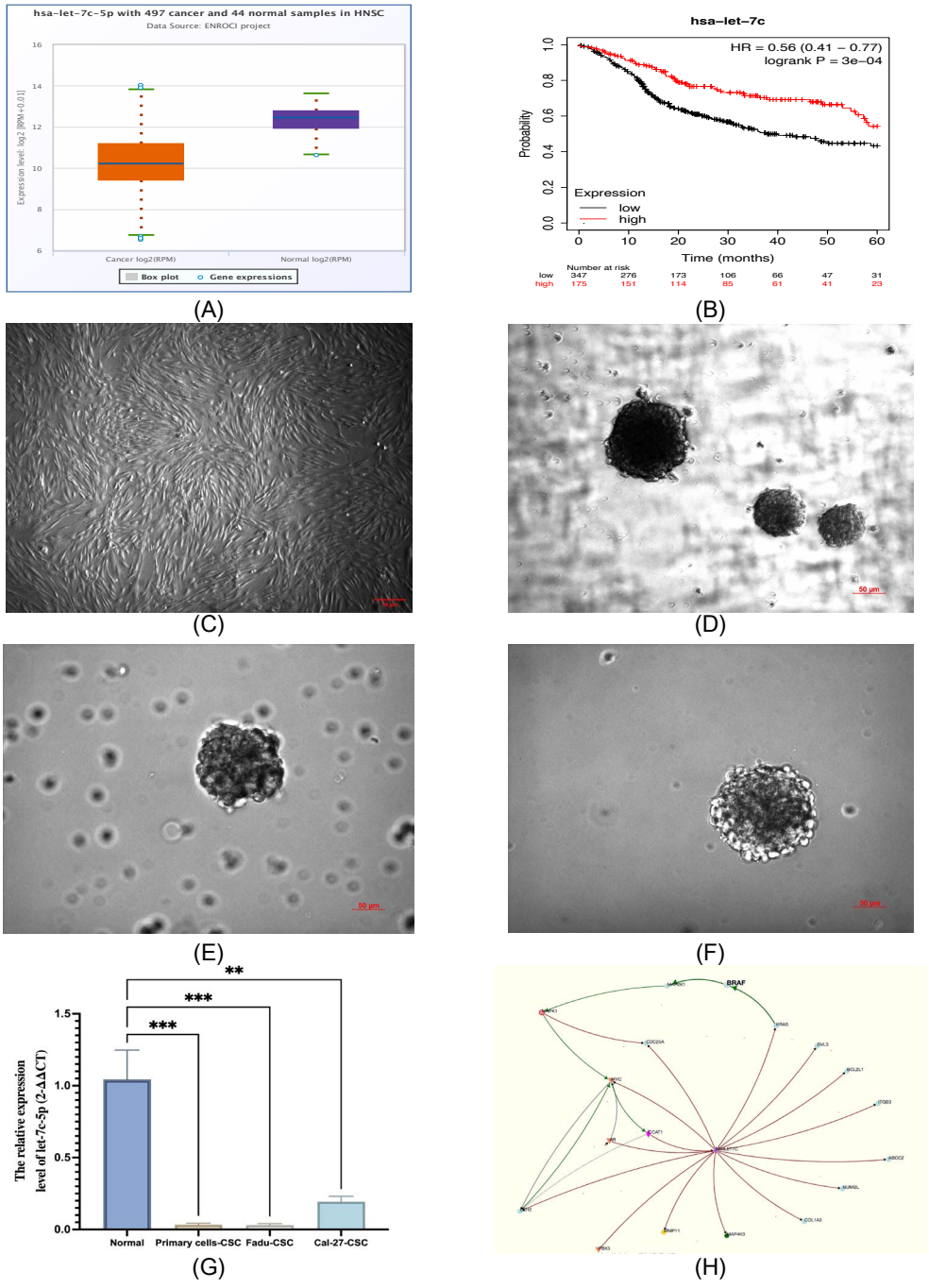

Figure 2. (A) Box Plot of Let-7c-5p Expression in HNSCC, (B) Survival Curve Plot of Let-7c Expression in HNSCC, (C) Morphology of Primary normal cell line(40x), (D) Morphology of FADU-CSC (40x), (E) Morphology of CAL27-CSC (40x), (F) Morphology of Primary cancer cell -CSC (40x), (G) Expression of let-7c-5p in Normal paired non-cancerous cells and Primary cancer cells-, Fadu-, Cal-27-induced CSC (***P<0.001, ** P<0.01), (H) A network of the interaction of targeted genes of let-7c-5p.

Table 2. Performance of 15 targeted genes of let-7c-5p in regulating cancer biological processes.

Process	Number	Up-regulation genes	Down-regulation genes
Migration	9	AR, CCAT1	MYC, EZH2, PBX3, DVL3, ITGB3, MAP4K3, MMP11
Invasion	9	AR	MYC, EZH2, PBX3, DVL3, ITGB3, MAP4K3, MMP11, KRAS
Metastasis	10	AR	MYC, EZH2, COLA1A2, PBX3, DVL3, ITGB3, MAP4K3, MMP11, KRAS
Tumor growth	8	AR	MYC, EZH2, PBX3, KRAS, MMP11, NUMBL, CDC25A
Cell spreading	6	AR	MYC, EZH2, PBX3, KRAS, MMP11
Apoptosis	5	AR, CCAT1	MYC, EZH2, CDC25A
Cisplatin resistance	5	AR	MYC, EZH2, BCL2L1, ABCC2
Stemness	4	AR	MYC, EZH2, NUMBL

Table 3. 17 selected targeted signaling pathways by 15 targeted genes of let7c-5p in HNC according to the high P value.

Rank	Pathway	P value	Genes
1	MicroRNAs in cancer	2.29E-06	ITGB3; MYC; KRAS; CDC25A; EZH2
2	Chemical carcinogenesis	2.4474E-05	AR; MYC; KRAS; CDC25A
3	Pathways in cancer	3.118E-05	AR; MYC; DVL3; KRAS; BCL2L1
4	PI3K-Akt signaling pathway	0.00011287	ITGB3; MYC; KRAS; BCL2L1
5	Signaling pathways regulating pluripotency of stem cells	0.00015291	MYC; DVL3; KRAS
6	Cellular senescence	0.0001977	MYC; KRAS; CDC25A
7	Transcriptional misregulation in cancer	0.00036398	MYC; PBX3; BCL2L1
8	Proteoglycans in cancer	0.00044088	ITGB3; MYC; KRAS
9	Notch signaling pathway	0.00087639	NUMBL; DVL3
10	Mitophagy	0.00116227	KRAS; BCL2L1
11	Central carbon metabolism in cancer	0.0012311	MYC; KRAS
12	MAPK signaling pathway	0.00125489	MYC; KRAS; MAP4K3
13	Human papillomavirus infection	0.00176316	ITGB3; DVL3; KRAS
14	ErbB signaling pathway	0.00180812	MYC; KRAS
15	Cell cycle	0.00379776	MYC; CDC25A
16	Autophagy	0.00461338	KRAS; BCL2L1
17	Apoptosis	0.00494684	KRAS; BCL2L1

Table 4. The prediction of FDA-approved drugs related to the KRAS and BCL2L1 of mitophagy pathways according to the highest P value.

Rank	Term	P-value	Genes
1	Lonafarnib	5.00E-04	KRAS
2	Vemurafenib	6.00E-04	KRAS
3	Dabrafenib	9.00E-04	KRAS
4	Gossypol	0.00129958	BCL2L1
5	Methysergide	0.00239858	BCL2L1

research over the past two decades (Ilieva et al., 2022). Several miRNAs, including miR-483-5p, miR-200, and miR-10, participated in the roles of CSCs or mitochondria, causing drug resistance and tumor recurrence in HNC(Fan et al., 2015; Huang et al., 2020; Yang J, 2020). However, the unique miRNAs for eliminating CSCs through the mitochondrial pathway to suppress drug resistance and tumor recurrence in HNC have not been reported. In this study, let-7c-5p was identified as a potential miRNA in HNC associated with mitochondria, cancer stem cells, drug resistance, and tumor recurrence by bioinformatics and experiments.

Let-7c-5p was one of the let-7 family members, generally recognized as a tumor suppressor that inhibits diverse oncogenes (Lee et al., 2016). Let-7c-5p has been reported to act in several biological mechanisms, just as in other malignancies, to predict and prevent drug resistance, stemness, and tumor recurrence. Let-7c-5p in HNC targets p53, CDK4, and IL-8 to suppress cell proliferation, drug resistance, and recurrence via histone acetylation, apoptosis, and stemness processes (Jin et al., 2017). This study uses bioinformatics and data mining analysis to identify how let-7c-5p interacts with target mRNAs to regulate drug resistance, stemness, and recurrence features. Based on the findings, let-7c-5p may target 15 mRNAs to regulate several critical biological processes of cancer, including apoptosis, cisplatin resistance, and stemness. The three main factors in 15 targets were androgen receptor (AR), MYC proto-oncogene (MYC), and enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2). In HNC, AR decreases as a transcription factor, MYC increases as a transcription factor, and EZH2 increases as an mRNA(Hu et al., 2020; Mehdi et al., 2022; Woods et al., 2022). Meanwhile, we draw a network of interactions of let-7c-5p and its targets by data mining tools, which might rapidly enable us to investigate the roles of let-7c-5p in recurrent HNC and its potential target in therapy.

As we know, CSCs play an essential role in tumor recurrence due to their drug resistance and stemness features, which make them more resistant to apoptosis than normal cancer cells (Picon & Guddati, 2021). Based on our results and literature review, let-7c-5p affects the apoptosis process by regulating AR, CCAT1, MYC, EZH2, and CDC25A, which is the mechanism related to cell cycle and cell DNA damage (Wang et al., 2021). Moreover, let-7c-5p modulates the mechanism involved in cisplatin transport and DNA damage, including AR, MYC, EZH2, ABCC2, and BCL2L1, to influence cisplatin resistance (Hill et al., 2019; Yue et al., 2023). Interestingly, NUMBL, a target gene of let-7c-5p, preserves stemness by participating in EMT, an essential biological process that induces drug resistance (Ebrahimie et al., 2021). Hence, let-7c-5p would regulate the genes involved in the EMT and drug transport process to preserve the stemness and drug resistance feature of CSCs. This would regulate CSC apoptosis by suppressing the DNA damage process, which is the reason for the HNC recurrence.

Further, enrichment analysis of let-7c-5p's target genes was used to explore the roles of mitochondrial function of head and neck cancer stem cells in drug resistance and recurrence. The findings demonstrated a link between cancer and the mitophagy pathway, which KRAS and BCL2L1 regulate. The mitochondrial function in CSCs was flexible, depending on the tumor types, environmental stimuli in the experimental system, and dynamic cellular phenotypes, including the transition from quiescent to proliferative CSCs (Huang et al., 2020). However, it was determined that mitophagy is the primary survival response mechanism for CSC development, reproduction, and tumorigenicity (Lin et al., 2021). Meanwhile, increasing evidence links mitophagy to stemness, dormancy, and drug resistance (Smith & Macleod, 2019). KRAS4A and KRAS4B are two subtypes of the KRAS oncogene activated by hypoxia and ER stress, respectively. It was more abundant in cancer stem cells and linked to eliminating chemo-induced mitophagy inhibition (Chen et al., 2019). One of the BCL2 family members, BCL2L1, is a critical antiapoptotic molecule that controls both mitochondrial apoptosis and autophagy and can participate in mitophagy in response to hypoxia (Wu et al., 2014). Therefore, let-7c-5p might participate in mitophagy by targeting KRAS and BCL2L1 to preserve the survival of CSCs.

Notably, the PI3K-Akt signaling pathway, one of the most critical aberrantly-activated pathways in cancer that is associated with chemotherapy resistance, apoptosis, and autophagy, was also highly enriched in our result by both KRAS and BCL2L1 (Hennessy et al., 2005; Stark et al., 2015). This also explained why both of them enriched the apoptosis and autophagy pathways.

The development of targeting PI3K pathway drugs showed good prospects in solid tumors and hematological tumors. However, they also faced many issues, such as drug resistance and drug toxicity (Vanhaesebroeck et al., 2021). Thus, the IDG database helped us predict the most potential drugs that will target KRAS and BCL2L1, including lonafarnib, vemurafenib, dabrafenib, gossypol, and methysergide. They all show anticancer potential in various solid tumors linked to KRAS, including lonafarnib (farnesyl transferase inhibitor), vemurafenib, and dabrafenib (BRAF kinase inhibitors) (Appels et al., 2011; Falchook et al., 2012; Subbiah et al., 2019). In addition, methysergide did not show any antitumor potential, and Gossypol, a BCL2L1 inhibitor, has been investigated for its anticancer activity (Wu et al., 2014). However, they also faced challenges, such as drug resistance, high cytotoxicity, and unselective activity (Hongnak & Gust, 2023; Pal et al., 2022). Therefore, the miRNA-based therapeutic approach may provide novel solutions to the abovementioned challenges.

CONCLUSION

Bioinformatics, experiment, and data mining analysis results showed that let-7c-5p may regulate HNC drug resistance and recurrence properties via the mitochondria and CSC process. Let-7c-5p and its target genes have an interaction network to participate in the cancer biological process, and let-7c-5p may specifically target KRAS and BCL2L1 to regulate mitophagy and apoptosis pathways for preserving cancer stem cells alive. Drug resistance and tumor recurrence in HNC may be addressed creatively by the let-7c-5p-based therapy approach. However, the predictions should be verified by more experimental studies.

ACKNOWLEDGMENTS

The authors would like to give a special thanks to the late Associate Professor Dr. Norhafizah Mohtarrudin from the Department of Pathology, Universiti Putra Malaysia, Serdang, Selangor, Malaysia for her contributions to design and supervision of this study.

FUNDING STATEMENT

This study was funded by the Fundamental Research Grant Scheme (FRGS/1/2020/SKK06/UPM/01/2), Ministry of Higher Education, Malaysia, and Universiti Putra Malaysia Research University Grant Scheme (GP-IPS/2022/9724000).

CONFLICT OF INTERESTS

The authors declare that they have no competing interests.

AUTHORS CONTRIBUTIONS

XZ and WLT conducted the bioinformatics analysis and experiment. YKC, XZ, and STS designed this study. YKC, STS, and VHF contributed to the supervision and discussion. XZ and YKC wrote and edited the manuscript. All authors read and approved the final manuscript.

REFERENCES

Appels NMGM, Bolijn MJ, van Eijndhoven MAJ, Stephens TC, et al (2011). Characterization of the in vitro activity of AZD3409, a novel prenyl transferase inhibitor. *Cancer Chemotherapy and Pharmacology*. 67: 137–145.

Fu CHEN, Zheng LAO, Zhang HY, Jie WANG, et al. (2022). Elevation of miR-125b-5p is related to improved prognosis in laryngeal squamous cell carcinoma and inhibits the malignancy and glycometabolic disorder by targeting MAP3K9. *Neoplasma*, 69: 3.

Chen WC, To MD, Westcott PMK, Delrosario R, et al. (2019). Regulation of KRAS4A/B splicing in cancer stem cells by the RBM39 splicing complex. *bioRxiv*: 646125.

Cree IA (2011). Principles of cancer cell culture. Cancer Cell Culture: Methods and Protocols. pp. 13-26.

Ebrahimie E, Rahimirad S, Tahsili M, Mohammadi-Dehcheshmeh M, (2021). Alternative RNA splicing in stem cells and cancer stem cells: Importance of transcript-based expression analysis. *World Journal of Stem Cells*. 13: 1394–1416.

Falchook GS, Long GV, Kurzrock R, Kim KB, et al. (2012). Dabrafenib in patients with melanoma, untreated brain metastases, and other solid tumours: A phase 1 dose-escalation trial. *The Lancet*. 379: 1893–1901.

Fan S, Chen WX, Lv XB, Tang QL, et al. (2015). miR-483-5p determines mitochondrial fission and cisplatin sensitivity in tongue squamous cell carcinoma by targeting FIS1. *Cancer Letters*. 362: 183–191.

Goldenberg D, Lee J, Koch WM, Kim MM, et al. (2004). Habitual Risk Factors for Head and Neck Cancer. *Otolaryngology–Head and Neck Surgery*. 131: 986–993.

Hennessy BT, Smith DL, Ram PT, Lu Y, et al. (2005). Exploiting the PI3K/AKT pathway for cancer drug discovery. Nature Reviews. *Drug Discovery* 4: 988–1004.

Hill DP, Harper A, Malcolm J, McAndrews MS, et al. (2019). Cisplatin-resistant triple-negative breast cancer subtypes: Multiple mechanisms of resistance. *BMC Cancer* 19: 1039.

Hongnak S and Gust R (2023). Structure-activity relationship study to improve cytotoxicity and selectivity of lonafarnib against breast cancer cells. *Archiv Der Pharmazie*. Pp: e2200263.

Hu C, Fang D, Xu H, Wang Q, et al. (2020). The androgen receptor expression and association with patient's survival in different cancers. *Genomics* 112: 1926–1940.

Huang T, Song X, Xu D, Tiek D, et al. (2020). Stem cell programs in cancer initiation, progression, and therapy resistance. *Theranostics*. 10: 8721–8743.

Ilieva M, Panella R, Uchida S, (2022). MicroRNAs in Cancer and Cardiovascular Disease. *Cells.* 11: 3551.

Jin L, Miao J, Liu Y, Li X, et al. (2017). Icaritin induces mitochondrial apoptosis by up-regulating miR-124 in human oral squamous cell carcinoma cells. *Biomedicine & Pharmacotherapy*. 85, 287–295.

Lee H, Han S, Kwon CS, Lee D, (2016). Biogenesis and regulation of the let-7 miRNAs and their functional implications. *Protein & Cell* 7: 100–113.

Lin Q, Chen J, Gu L, et al. (2021). New insights into mitophagy and stem cells. *Stem Cell Research & Therapy*. 12: 452.

Lo YL, Wang CS, Chen YC, et al. (2020). Mitochondrion-Directed Nanoparticles Loaded with a Natural Compound and a microRNA for Promoting Cancer Cell Death via the Modulation of Tumor Metabolism and Mitochondrial Dynamics. *Pharmaceutics*. 12: 756.

Lopez J and Tait SWG (2015). Mitochondrial apoptosis: Killing cancer using the enemy within. *British Journal of Cancer.* 112: 957–962.

Lth P, In S, Yg Y, et al. (2018). Cancer Stem Cells (CSCs) in Drug Resistance and their Therapeutic Implications in Cancer Treatment. *Stem Cells International*. 2018.

Marur S, Forastiere AA (2008). Head and Neck Cancer: Changing Epidemiology, Diagnosis, and Treatment. *Mayo Clinic Proceedings*. 83: 489–501.

Mehdi AM, Zhou C, Turrell G, et al. (2022). HPV status represents dominant trait driving delineation of survival-associated gene co-expression networks in head and neck cancer. *Cancer Gene Therapy*. Pp: 1–12.

Mody MD, Rocco JW, Yom SS, et al. (2021). Head and neck cancer. *The Lancet.* 398: 2289–2299.

Pal D, Sahu P, Sethi G, et al. (2022). Gossypol and Its Natural Derivatives: Multitargeted Phytochemicals as Potential Drug Candidates for Oncologic Diseases. *Pharmaceutics.* 14: 2624.

Picon H and Guddati AK (2021). Cancer stem cells in head and neck cancer. *American Journal of Stem Cells* 10: 28–35.

Singh P, Augustine D, Rao RS, et al. (2021). Role of cancer stem cells in head-and-neck squamous cell carcinoma—A systematic review. *Journal of Carcinogenesis* 20: 12.

Smith AG, Macleod KF. (2019). Autophagy, cancer stem cells and drug resistance. *The Journal of Pathology* 247: 708–718.

Stark AK, Sriskantharajah S, Hessel EM, et al. (2015). PI3K inhibitors in inflammation, autoimmunity and cancer. *Current Opinion in Pharmacology*. 23: 82–91.

Subbiah V, Gervais R, Riely G, et al. (2019). Efficacy of vemurafenib in patients with non–small-cell lung cancer with BRAF V600 mutation: an open-label, single-arm cohort of the histology-independent VE-BASKET study. *JCO Precision Oncology*. 3: 1-9.

Sung H, Ferlay J, Siegel RL, et al. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians*. 71: 209–249.

Torre-Healy LA, Berezovsky A, Lathia JD, (2017). Isolation, characterization, and expansion of cancer stem cells. *Adult Stem Cells: Methods and Protocols*. 133-143.

Vanhaesebroeck B, Perry MWD, Brown JR, et al. (2021). PI3K inhibitors are finally coming of age. *Nature Reviews. Drug Discovery*. 20: 741–769.

Wang TH, Chen CC, Leu YL, et al. (2021). Palbociclib induces DNA damage and inhibits DNA repair to induce cellular senescence and apoptosis in oral squamous cell carcinoma. *Journal of the Formosan Medical Association* 120: 1695–1705.

Woods ML, Weiss A, Sokol AM, et al. (2022). Epigenetically silenced apoptosis-associated tyrosine kinase (AATK) facilitates a decreased expression of Cyclin D1 and WEE1, phosphorylates TP53 and reduces cell proliferation in a kinase-dependent manner. *Cancer Gene Therapy* 29: 1975-1987.

Wu H, Xue D, Chen G, et al. (2014). The BCL2L1 and PGAM5 axis defines hypoxia-induced receptor-mediated mitophagy. *Autophagy*. 10: 1712–1725.

Wx Z, Rabinowitz JD, White E, (2016). Mitochondria and cancer. *Molecular cell* 61: 667-676.

Yang J, Wu SP, Wang WJ, et al. (2020). A novel miR-200c/c-myc negative regulatory feedback loop is essential to the EMT process, CSC biology and drug sensitivity in nasopharyngeal cancer. *Experimental Cell Research*. 391: 111817.

Yue P, Han B, Zhao Y, (2023). Focus on the molecular mechanisms of cisplatin resistance based on multi-omics approaches. *Molecular Omics*. 19: 297-307.