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Original Article

A comprehensive bioinformatics analysis of fatty acid metabolism-associated genes in the diagnosis and prognosis of head and neck squamous cell carcinoma

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Abstract

Background and purpose: One of the most prevalent types of malignancies affecting the cells in the mucosal surface of the oral cavity and pharynx regions is head and neck squamous cell carcinoma (HNSCC). This study analyzed the metabolic profile of genes involved in the metabolism of fatty acids (FAs) to identify biomarkers with prognostic and diagnostic potential in HNSCC.
Experimental approach: Gene set enrichment analysis, differential gene expression, and correlation analysis methods were used to examine the enrichment and expression patterns of genes involved in the metabolism of FAs in the HNSCC tissue samples. Gene ontology and network analysis were performed to explore the molecular interactions in the metabolic pathways of FAs. The diagnostic and prognostic potentials of identified highly dysregulated genes in HNSCC were examined by ROC test and Cox-regression methods.
Findings/Results: FA-associated metabolic pathways were significantly dysregulated in the HNSC cancer samples. For the diagnosis of HNSC cancer, CYP4B1 and FMO2 could be potential biomarkers, while for the prognosis of HNSCC survival periods, ACOX2, CYP4F12, and ELOVL6 could hold valuable biomarker potential.
Conclusion and implications: The findings could help target the metabolism of FAs using the identified biomarkers for the design of new therapeutic opportunities for patients with HNSCC.

Keywords: CYP4B1; Diagnosis; ELOVL6; Fatty acid metabolism; FMO2; Head and neck squamous cell carcinoma; Prognosis.

INTRODUCTION

The head and neck cancers (HNCs), as the 7th most common type of cancer with a high diagnosis rate globally, arise from the lining of cells in the mucosal layer of the larynx, pharynx, and oral cavity regions. Each year, more than 900,000 cases are reported to be diagnosed with HNCs. The low survival periods and unknown molecular background of HNCs have influenced the need for further investigation on HNCs (1-3). The metabolic profile of a variety of cancers has shown a great diversity and alterations at transcriptional and translational levels concerning the type and etiology of cancer cells.

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The metabolism of fatty acids (FAs) is one of the critical parts of cancer cell metabolism that highly impacts the optimal productivity, membrane integrity, cellular development, and survival of the cancer cells (4-6).

FAs are an important part of cellular metabolic building blocks that are highly used by cancer cells due to their initial role in the synthesis of lipid molecules, regulation of cellular signaling pathways, and energy production that aids cellular growth and development. The metabolism of FAs includes a wide range of enzymatic reactions, including the biosynthesis, elongation, modification, activation, catabolism, and homeostasis of FAs in cells, which all play a vital role in the progression and survival of cancer cells (7,8).

The metabolic profile of tumor cells can change according to environmental conditions and cellular growth rate, and the regulation of homeostasis and metabolism FAs is critical for the optimal progression of cancer cells. Therefore, a clear understanding of the interconnectivity and the expression profile of FA-associated metabolic pathways could provide useful insights for the development of new personalized therapeutic approaches that rely on targeting the metabolism of FAs based on the metabolic profile of each patient diagnosed with cancer (9-12).

Multiple studies have investigated the impact of fatty acid oxidation (FAO) and fatty acid metabolism (FAM) in the oncogenesis, progression, and development of human cancer cells, such as liver, colorectal, and breast cancer. Recent investigations have highlighted the impact of FAO in cancer cells, particularly in the development of drug resistance. FAO has been found to support the synthesis of cellular DNA in endothelial cells, indicating that the inhibition of the FAO pathway could potentially help with inhibiting tumor angiogenesis by targeting the endothelial cells in blood vessels that nourish tumor cells (4,13,14). These findings suggest that blocking FAO could be a promising strategy to overcome drug resistance and hinder tumor growth (13). Multiple genes, including fatty acid synthase (FASN), ATP citrate lyase (ACLY), and acetyl-CoA carboxylase (ACC), involved in the metabolism of FAs, have been identified as effective targets for inhibiting cancer progression (15). By disrupting the metabolism of FAs in cancer cells, it is possible to potentially inhibit their growth and reduce the risk of resistance (16). However, further research is needed to fully understand the complexities of FAM in different cancer types and develop effective therapies that can be converted into clinical practice (17). While multiple studies have reported a dysregulated expression pattern in the cellular metabolic pathways in the head and neck squamous cell carcinoma (HNSCC) (18-21), there is a great lack of knowledge regarding the expression profile of molecular pathways involved in the metabolism of FAs in HNSCC patients.

In the current study, we analyzed the expression profile of HNSCC tumor and control tissue samples to identify the enrichment pattern and expression profile of genes involved in varying pathways that participated in the metabolism of FAs by the help of bioinformatics, and the interaction between these genes and their molecular functions was predicted as well. Based on the expression patterns of HNSCC patients, we introduced high-potential biomarkers for the diagnosis and prognosis of HNSCC that could encourage future investigations for the development of better personalized treatment approaches.

MATERIALS AND METHODS

Data processing and gene expression analysis

The RNA-seq count data of 502 HNSCC tissue samples along 44 normal adjacent tissue samples were downloaded from the Cancer Genome Atlas (TCGA) online platform (www.doc.gdc.cancer.goc/), concerning the principles of Helsinki. The count data were converted into logarithmic format after normalization with the Voom package and subjected to differential gene expression analysis with the help of TCGAbiolinks, Limma, and edgeR packages in R programming software (Version 4.2.3) to identify the most differentially expressed genes (DEGs) involved in the FAM of HNSCC (22-24). The clinical information of the TCGA HNSCC patients has been summarized in Table S1. For further validation of the differential gene expression

results, the expression data of GSE58911 with 15 HNSC cancer samples and 15 normal tissue samples were downloaded from the Gene Expression Omnibus (GEO) dataset (https://www.ncbi.nlm.nih.gov/geo) and analyzed using the GEO2R tool (https://www.ncbi.nlm.nih.gov/geo/geo2r/) (25).

Gene set enrichment analysis

Gene set enrichment analysis (GSEA) is a commonly used approach for the estimation of correlation ratio between the expression levels of a specific gene set that plays a common molecular function in a biological pathway in the interest disease phenotype. This analysis was performed using the GSEA software (version 4.0.3) developed by the Broad Institute, with 5 different gene sets downloaded from MSigDB (www.gsea-msig.org/) that were associated with the metabolic reactions of FAs, such as the elongation, catabolism, homeostasis, biosynthesis, and the metabolism of FAs (26,27). The normalized expression data of HNSCC samples were utilized along with normal adjacent tissues as the expression dataset for GSEA. The t-test statistical approach was set for the calculation of metric ranking scores based on the correlation of gene expression levels and defined sample phenotypes, and the rest of the parameters were set as default.

Table S1. Summary statistics and distribution of variables in the study population. Descriptive statistics were elucidated in terms of the median with its corresponding interquartile range (IQR) for numeric variables, while categorical variables were conveyed by their respective frequencies and associated percentages.

Variables	Levels	Median (IQR)/frequency (%)
Days to last follow-up	-----	616.15 (171.75, 847.25)
Days to death	-----	747.85 (215.50, 800.25)
Tobacco smoking history	-----	2.46 (2.00, 4.00)
Year of tobacco smoking onset	-----	1967.31 (1959.00, 1975.00)
Stopped smoking year	-----	1997.25 (1989.75, 2009.00)
Number pack years smoked	-----	45.75 (25.00, 60.00)
Amount of alcohol consumption per day	-----	3.24 (0.00, 5.00)
Anatomic neoplasm subdivision	Alveolar ridge	18 (3.41)
	Base of tongue	27 (5.11)
	Buccal mucosa	23 (4.36)
	Floor of the mouth	63 (11.93)
	Hard palate	7 (1.33)
	Hypopharynx	10 (1.89)
	Larynx	117 (22.16)
	Lip	3 (0.57)
	Oral cavity	73 (13.83)
	Oral tongue	133 (25.19)
	Oropharynx	9 (1.70)
	Tonsil	45 (8.52)
Gender	Male	386 (73.11)
	Female	142 (26.89)
Vital status	Alive	358 (67.80)
	Dead	170 (32.20)
Clinical stage	Stage I	21 (3.98)
	Stage II	99 (18.75)
	Stage III	107 (20.27)
	Stage IVA	269 (50.95)
	Stage IVB	11 (2.08)
	Stage IVC	7 (1.33)
	NA	14 (2.65)
	[Not evaluated]	114 (21.59)
	[Unknown]	8 (1.52)
HPV status	Negative	74 (14.02)
	Positive	41 (7.77)
	NA	291 (55.11)
Alcohol history documented	No	165 (31.25)
	Yes	352 (66.67)
	NA	11 (2.08)

Gene expression and correlation analysis

To clarify the expression patterns of the genes involved in the metabolism pathways of FAs with significant enrichment in HNSCC tissues, differential gene expression analysis was performed. The log2 expression levels and log2 of fold-change values were calculated for the top 10 genes with the highest metric ranking scores, and graphs were created using the GraphPad Prism software (version 9.1.0). For better understanding the correlation between the expression levels of best enriched genes in FAM pathways, correlation analysis was performed using the metan package in R programming software, following the Pearson statistical method for the calculation of correlation scores between the selected genes. The *P*-values < 0.01 were considered statistically notable for assessing differential gene expression levels (23,24,26,28,29).

Gene ontology and protein-protein interaction analysis

To achieve a better perspective over the molecular interactions between genes involved in FAs- FAs-associated metabolic pathways, the protein-protein interaction (PPI) network analysis was performed using the STRING database (www.string-db.org, version 10) (30,31) available in Cytoscape software (www.cytoscape.org, version 3.2.0) (32,33), and the threshold of interaction scores was set to a confidence range of 0.70. The best interactive genes in the generated PPI network were analyzed based on degree scores calculated with the CytoNCA application available in Cytoscape software (www.apps-cytoscape.org/apps/cytocna) (34,35). The Gene Ontology (GO) is a freely available database widely used in bioinformatics to annotate gene functions, cellular locations, and biological processes, and researchers can use this database to analyze and interpret large-scale genomic data, such as gene expression profiles and protein interactions. The GO analysis was also done for all genes in the selected gene sets from the metabolic pathways of FAs to explore the most enriched molecular functions and pathways in which a large number of genes are involved. GO analysis was performed by the DAVID database (www.David-d.ncifcrf.gov, version 6.8) (36,37), and the generated results were ordered according to the largest count of genes to the smallest gene counts associated with each molecular and biological term.

Receiver operating characteristic test

To assess the diagnostic potential of the top metric-ranked genes from the metabolic pathways of FAs enriched in HNSCC samples, the receiver operating characteristic (ROC) curve was created based on the normalized expression data of the genes in HNSCC. The ROC curves were generated by GraphPad Prism software (version 9.1.0). The significance of the diagnostic power of genes was compared based on the generated area under the curve (AUC) and *P*-values.

Cox-regression analysis

The prognostic biomarker potential of the top-ranked genes was estimated in HNSCC patients with the help of the OncoLnc online database (www.oncolnc.org), which used the Cox-regression statistical approach to calculate the risk correlation between the gene expression levels and survival of HNSCC TCGA patients based on their clinical information. The prognostic capability of the top enriched genes in FAM pathways was compared based on the Cox-regression coefficient and log-rank *P*-values (38,39).

RESULTS

GSEA analysis of FA-associated pathways in HNSCC

A practical approach for analyzing the enrichment patterns of genes with molecular functions associated with FAM is GSEA analysis, which is based on the Kolmogorov-Smirnov statistical method that gives an overall view of the cumulative distribution of the genes based on their expression levels. The results of GSEA analysis could be compared based on the reported metric ranking scores or enrichment scores (ES), which were based on a permutation analytical approach that reported a false discovery rate (FDR) value as well. The cut-off

value for FDR q-values was set to 0.25, and the gene sets with FDR q-values below 0.25 were considered notably enriched. In this study, 5 different gene sets associated with FAM were selected for GSEA analysis in HNSCC. As shown in Fig. 1, all the gene sets including FA biosynthetic process (normalized enrichment score (NES) = -1.52, FDR = 0.016), FA catabolic process (NES = -2.03, FDR = 0.00), FA homeostasis pathway (NES = -0.90, FDR = 0.571), and FA metabolic process (NES = -1.96, FDR = 0.00) had a negative NES, except for the FA elongation process (NES = 0.57, FDR = 0.964) that demonstrated a positive NES. However, according to the FDR cut-off value, the FA biosynthetic process, FA catabolic process, and FA metabolic process were notably enriched in HNSC cancer samples. Genes including pyruvate dehydrogenase kinase 4 (PDK4) (ES = -8.86), carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) (ES = -11.59), acyl-CoA oxidase 2 (ACOX2) (ES = -8.87), cytochrome P450 family 4 subfamily F member 12 (CYP4F12) (ES = -10.92), ELOVL fatty acid elongase 6 (ELOVL6) (ES = -5.64), diacylglycerol O-acyltransferase 2 (DGAT2) (ES = -5.21), protein kinase AMP-activated catalytic subunit alpha 2 (PRKAA2) (ES = -8.31), cytochrome P450 family subfamily B member 1 (CYP4B1) (ES = -17.04), and flavin-containing dimethylaniline monooxygenase 2 (FMO2) (ES = -17.99) demonstrated the highest ES among the members of the gene sets and were selected for next analyses.

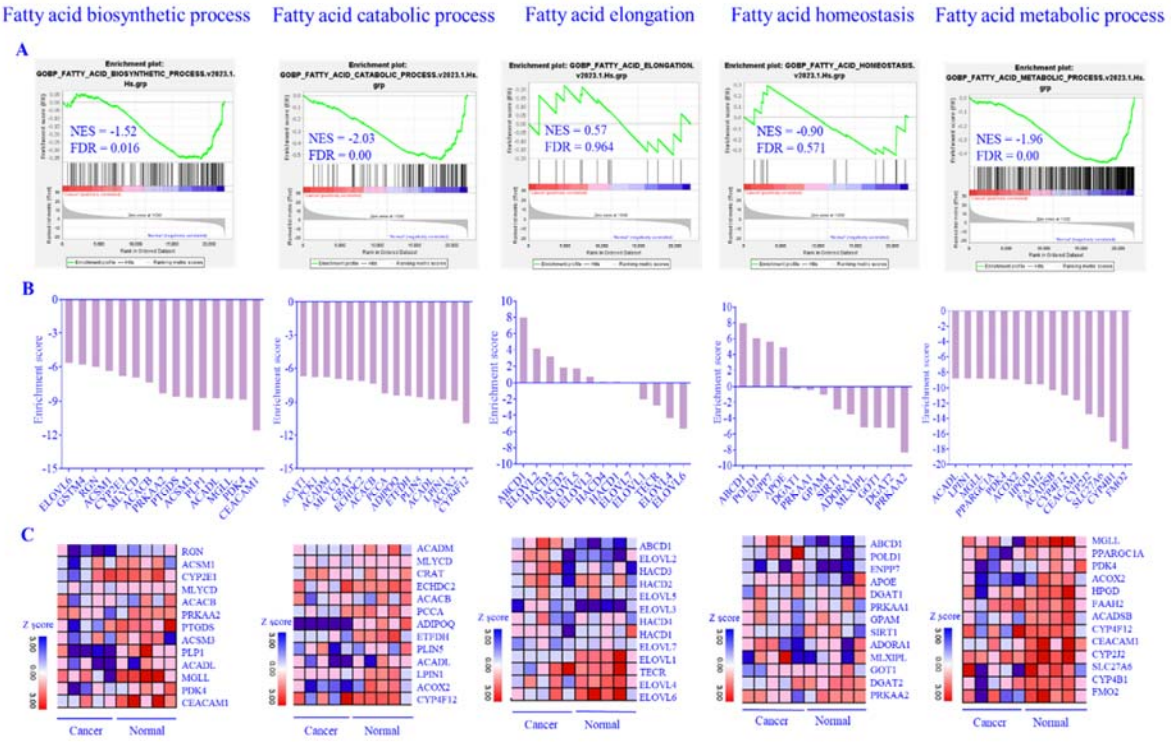

Fig. 1. GSEA of FA metabolism-associated pathways in head and neck squamous cell carcinoma. The enrichment pattern of gene sets involved in 5 different pathways associated with FA metabolism, catabolism, biosynthesis, homeostasis, and elongation processes was analyzed by the GSEA method using the normalized expression data of tissue samples from patients with HNSC cancer. Healthy tissues were considered the control group. (A) The enrichment plot, (B) score plots representing the ranked metric scores (tTest) obtained from GSEA analysis, and (C) heatmaps of enriched genes in cancer and normal tissue samples, colored according to the Z score. Gene sets with FDR q-values below 0.25 are considered significantly enriched. GSEA, Gene set enrichment analysis; FA, fatty acid; NES, normalized enrichment score; FDR, false discovery rate.

Downregulated expression pattern of FAM in HNSCC

The differential expression analysis of Log2 expression levels of the top-ranked genes from FA-associated metabolic pathways using TCGA samples, demonstrated the highest ES by GSEA analysis in the HNSCC tissues revealed that all of the genes except ATP binding cassette subfamily D member 1 (ABCD1) ($P < 0.001$) were downregulated significantly in HNSC cancer samples compared to normal tissue samples (Fig. 2). Two genes including CYP4B1 ($P < 0.001$) and FMO2 ($P < 0.001$) demonstrated the most down regulated levels compared to rest of the genes, such as CYP4F12 ($P < 0.001$) and PDK4 ($P < 0.001$) that also had relative significant down regulation in TCGA HNSC cancer samples (Fig. 2). The differential expression levels of the top-ranked genes were also checked in GSE29330 HNSC cancer samples compared to the paired normal tissue samples, and all the top-ranked selected genes from GSEA analysis showed notable negative expression levels in cancer samples, including ELOVL6 (Log2 FC = -0.189, $P = 0.003$), PDK4 (Log2 FC = -0.224, $P = 0.004$), ACOX2 (Log2 FC = -0.143, $P = 0.0005$), CYP4F12 (Log2 FC = -0.331, $P = 0.00006$), DGAT2 (Log2 FC = -0.099, $P = 0.01$), PRKAA2 (Log2 FC = -0.154, $P = 0.05$), CYP4B1 (Log2 FC = -0.30, $P = 0.0007$), FMO2 (Log2 FC = -0.28, $P = 0.02$), except CEACAM1 (Log2 FC = 0.013, $P = 0.720$), and ABCD1 (Log2 FC = -0.049, $P = 0.220$), which their expression level did not significantly differ between cancer and normal tissue samples.

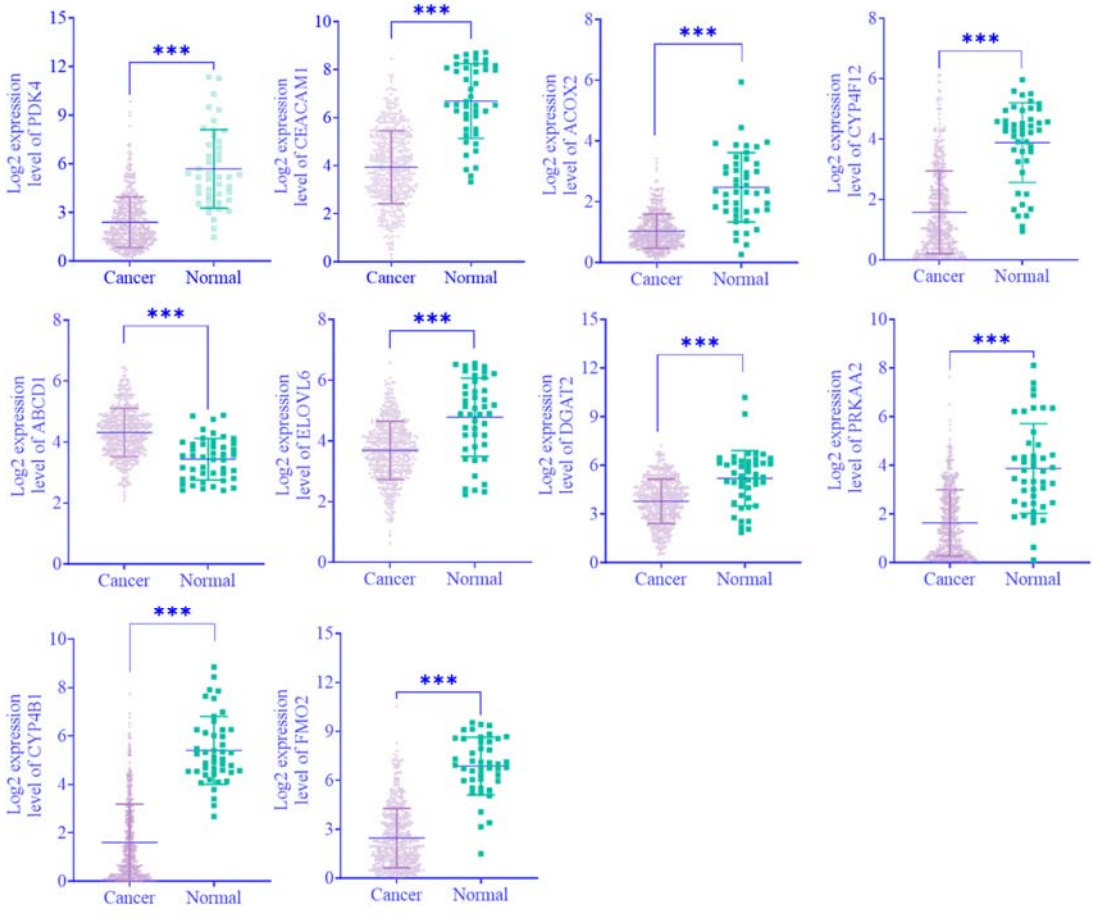

Fig. 2. Differential gene expression analysis of the top 10 enriched scored genes in fatty acid metabolic pathways in TCGA head and neck squamous cell carcinoma**.** Cancer and normal tissue samples were shown with red and blue colors, respectively. $P < 0.001$ revealed a significant difference between cancer and normal tissue samples.

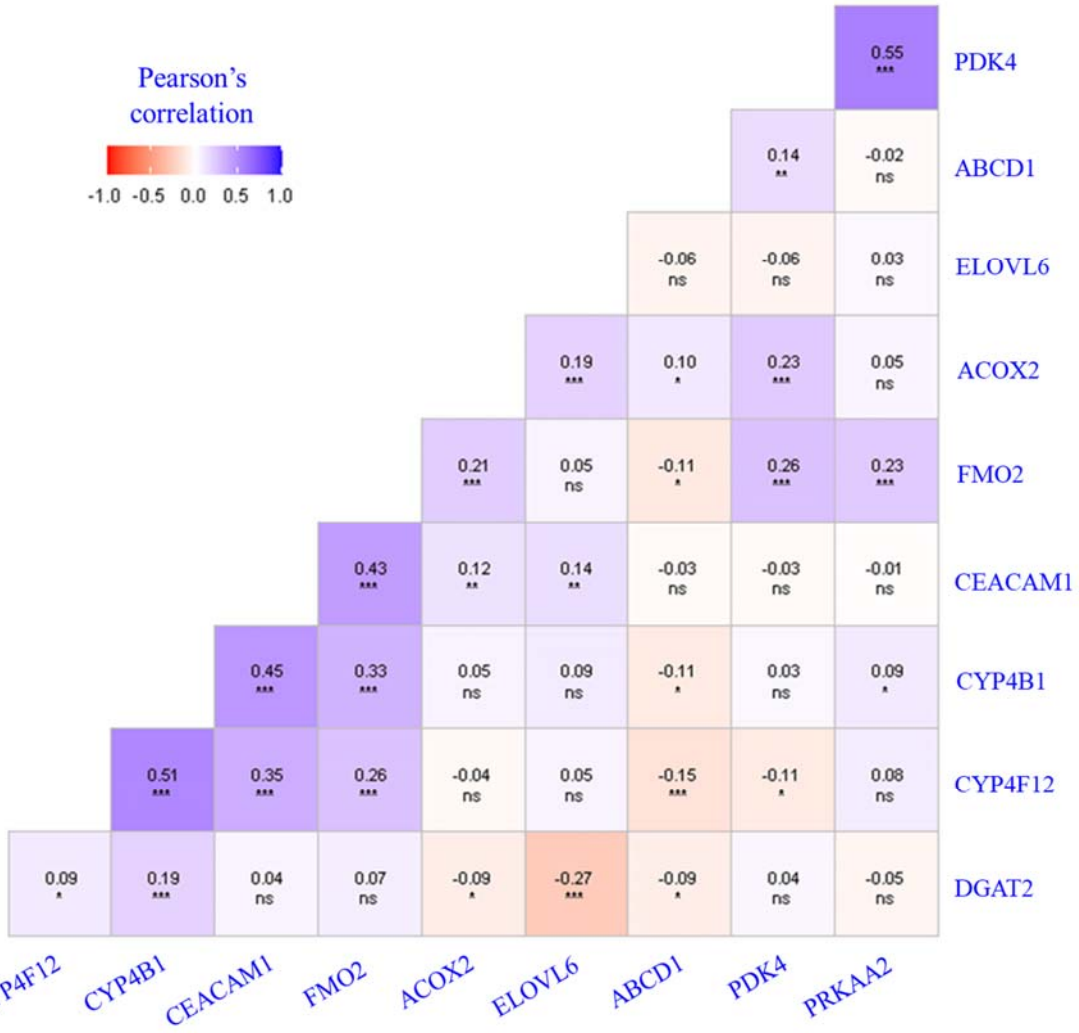

Fig. 3. Correlation analysis between top enriched genes from FA metabolism in HNSCC. The relationship between the expression levels of the top selected enriched genes from FA metabolic pathways in the HNSCC expression data were analyzed by Pearson correlation method. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ revealed significant differences. FA, Fatty acid; HNSCC, head and neck squamous cell carcinoma; ns, non-significant.

Genes that were grouped under similar biological pathways shared great connectivity and expressional correlations at the transcriptional level for the better maintenance of optimal cellular productivity. The pathways involved in the metabolism of FAs were also expected to co-regulate each other at the transcriptional level. To estimate the degree of correlation between the genes, correlation analysis was performed. As depicted in Fig. 3, the expression level of the CYP4B1 gene positively correlated with the expression levels of CYP4F12 (correlation coefficient = 0.51, P = 3.62 E-35) and CEACAM1 (correlation coefficient = 0.45, P = 2.63 E-26). The PDK4 expression level with PRKAA2 (correlation coefficient = 0.55, P = 6.60 E-41) correlated positively as well. A negative correlation was also detected between the expression levels of ELOVL6 and DGAT2 (correlation coefficient = -0.27, P = 1.12 E-09), which indicated that a possible negative regulatory mechanism at the transcriptional level might exist between the genes in HNSCC cells.

Identification of highly enriched pathways in FAM

The GSEA analysis of the selected gene sets involved in FAM using HNSCC expression data informs us about the enrichment patterns

of genes in each pathway, while giving no insight about the possible molecular interactions between the enriched genes. To achieve this insight, the PPI network was constructed using the complete gene list of all FAM pathways chosen for this study. As depicted in Fig. 4, all genes demonstrated notable interconnectivity with each other. The genes with higher co-expression values were colored orange, and genes with lower co-expression values estimated by the STRING database were colored blue. Also, the nodes that demonstrated the highest degree scores and interconnectivity have been colored pink according to the CytoNCA results. The topological parameters of the generated PPI network were assessed by the CytoNCA tool. Table 1 includes the top 20 interacting genes with the highest degree scores. The genes, including acyl-CoA oxidase 1 (ACOX1), peroxisome proliferator-activated receptor alpha (PPARA), acetyl-CoA acyltransferase 1 (ACAA1), FASN, and cytochrome P450 2E1 (CYP2E1) represented the best degree scores compared to the list of other genes involved in the metabolic pathways of FA. Also, ACOX1 and PPARA genes demonstrated the highest degree and betweenness scores compared to the rest of the genes, and the highest network score belonged to the ACOX1 gene. Therefore, it can be concluded that ACOX1 and PPARA play a notable role in the generated PPI network.

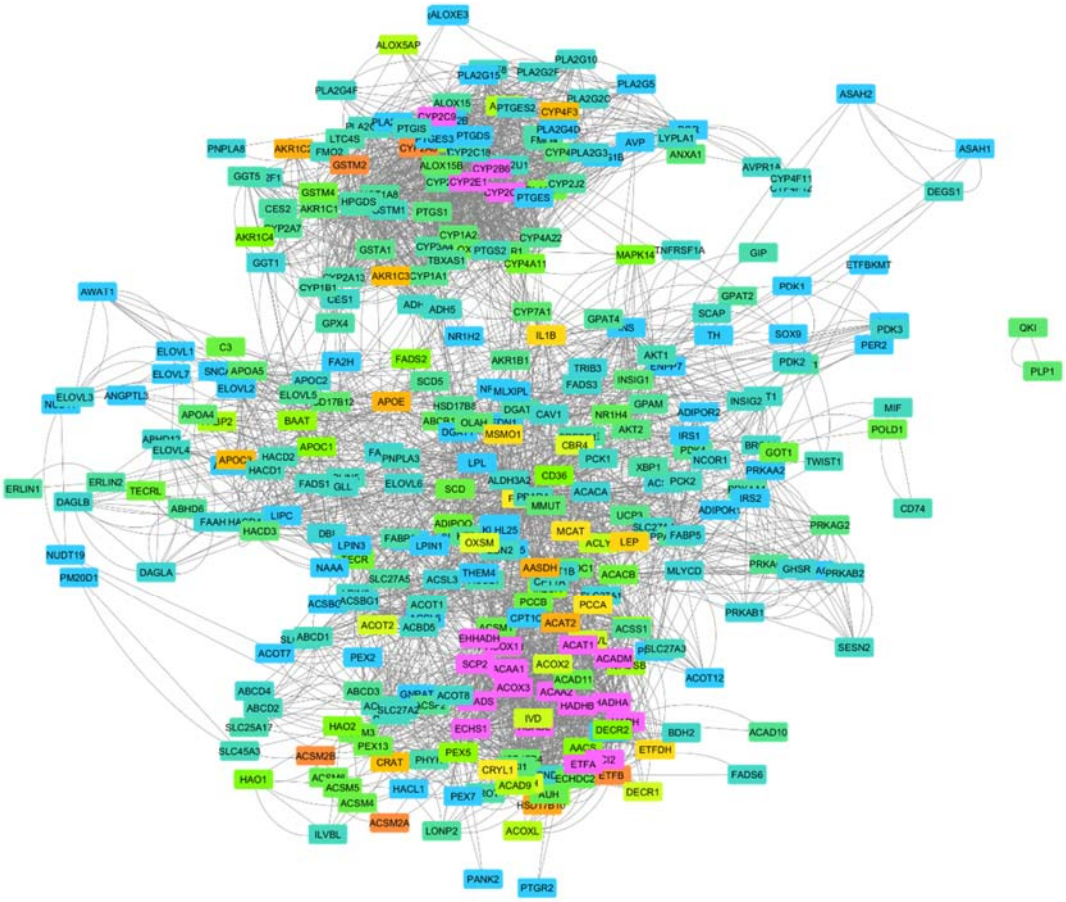

Fig. 4. PPI analysis between FAM-associated pathways. The PPI network was generated by the data retrieved from the STRING database into the Cytoscape software using the edge-weighted spring-embedded layout for the demonstration of the interconnectivity and interactions between the proteins involved in FAM. Genes have been colored based on their co-expression scores from blue to orange, and the top 20 genes that demonstrated the highest interconnectivity by CytoNCA application have been colored pink for better visualization. PPI, Protein-protein interaction; FAM, fatty acid metabolism.

Table 1. Top 20 genes with the highest degree scores in the protein-protein interaction network calculated by the CYTOCNA application.

Gene ID	Subgraph	Degree	Eigenvector	Betweenness	Closeness	Network
ACOX1	1.46 E+09	128	0.27	13366	0.12	85
PPARA	2.24 E+08	108	0.1	21456	0.12	48
ACAA1	1.00 E+09	96	0.22	4037	0.12	62
FASN	2.77 E+08	94	0.12	7719	0.12	47
CYP2E1	6.80 E+08	92	0.02	3343	0.11	67
SCP2	5.53 E+08	86	0.17	4181	0.12	49
ACOX3	9.11 E+08	80	0.21	3556	0.12	48
CYP3A4	4.15 E+08	76	0.02	3469	0.11	54
HADHB	8.32 E+08	74	0.2	873	0.11	51
CYP2C9	5.85 E+08	74	0.02	1238	0.11	55
PTGS2	3.47 E+08	74	0.02	8018	0.12	50
ACAA2	8.56 E+08	72	0.21	656	0.11	53
ACSL1	2.91 E+08	72	0.12	4569	0.12	33
ACACA	1.81 E+08	72	0.1	6393	0.12	35
PTGS1	3.30 E+08	70	0.02	1953	0.11	49
EHHADH	7.13 E+08	68	0.19	2610	0.12	41
ACADS	5.94 E+08	68	0.17	4543	0.11	39
CYP2B6	5.70 E+08	68	0.02	624	0.11	50
HPGDS	3.05 E+08	68	0.01	1381	0.11	47
SREBF1	8.24 E+07	66	0.06	3967	0.12	35

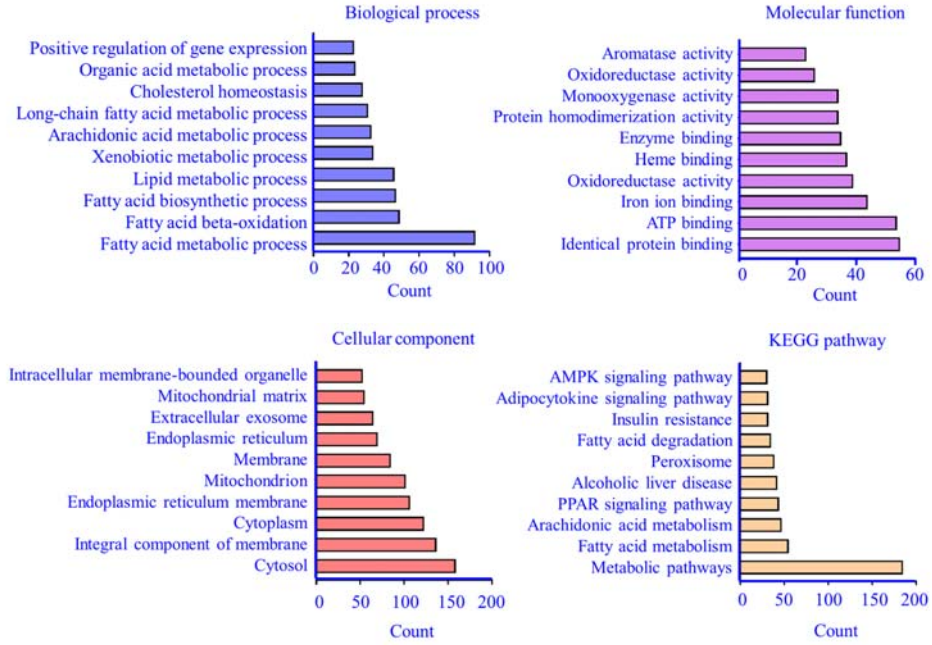

Fig. 5. Functional enrichment analysis of FA metabolism-associated genes. The Gene Ontology terms of the list of genes involved in FA metabolic pathways were retrieved from the DAVID database to identify the top-enriched biological processes and pathways with the largest gene counts involved. FA, Fatty acid.

To have a better understanding of the molecular functions and pathways that involve the largest number of genes from FAM gene sets, GO analysis was performed. As depicted in Fig. 5, more than 90 genes were predicted to be involved in fatty acid metabolic process (GO: 0006631), and the molecular functions of most of them were associated with identical protein binding (GO: 0042802), ATP binding (GO: 0005524), and iron binding (GO:0005506), which indicated the importance of these molecular functions in the metabolic pathways of FAs. The cellular component of a majority of the genes was predicted in cytosol (GO: 0005829), integral component of the membrane (GO: 0016021), and cytoplasm (GO: 0005737).

The Kyoto Encyclopedia of Genes and Genomes (KEGG) is a freely available database that provides useful information on metabolic pathways, regulatory networks, and molecular interactions, as well as the relationships between genes, proteins, and biological processes (40,41). The KEGG pathway analysis of the gene list suggested that a large number of genes participated in metabolic pathways (has01100), fatty acid metabolism (hsa01212), arachidonic acid metabolism (hsa00590), and peroxisome proliferator-activated receptor (PPAR) signaling (hsa03320).

Diagnostic potential of FA-associated genes in HNSCC

ROC curves are graphical approaches for the better comparison of the performance of various genes in the diagnosis and classification of cancer phenotype from normal tissue phenotype. For this test, the normalized expression data of the top-enriched genes in FAM were used to assess their diagnostic capability in the HNSCC. As shown in Fig. 6, all of the genes represented a notable diagnostic potential. However, CYP4B1 (AUC = 0.95) and FMO2 (AUC = 0.94) genes performed much stronger compared to the rest of the genes and could be good candidates for early diagnosis and screening of HNSC cancer.

Analysis of prognostic biomarker capability of FA-associated genes in HNSCC

The correlation between the expression levels of the top-enriched genes in FAM with the survival period of patients with HNSCC by the Cox-regression analysis method and Cox coefficient values along log-rank *P*-values was calculated with the OncoLnc database. As depicted in Fig .7, most of the selected genes from FAM demonstrated weak prognostic capability in HNSCC, except for ACOX2, CYP4F12, and ELOVL6 genes, which demonstrated significant prognostic potential in HNSCC. The prognostic potential of the ELOVL6 gene was most statistically significant compared to other genes, and it appeared that patients who had higher expression levels of the ELOVL6 gene survived for shorter periods compared to the low-expression group.

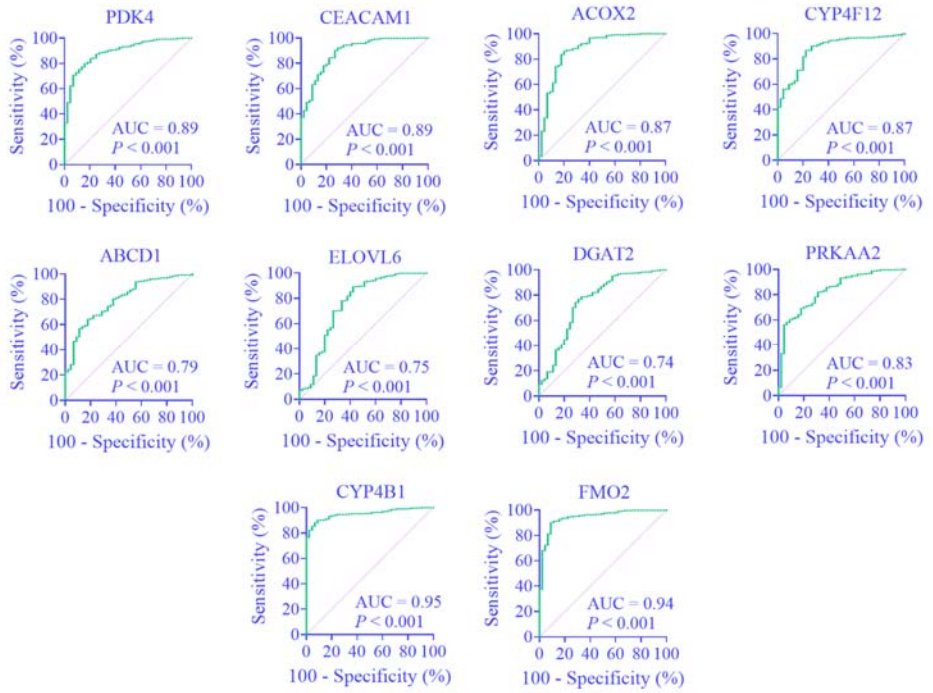

Fig. 6. ROC test of top enriched genes from FA metabolism in HNSCC**.** The diagnostic potential of the selected enriched genes from FA metabolic pathways was analyzed using their normalized expression data in the HNSCC tissue samples, and AUC values were generated by taking an ROC test with GraphPad Prism software. Two genes, including CYP4B1 and FMO2, demonstrated great diagnostic potential. $P < 0.001$ revealed a significant difference in each gene. ROC, Receiver operating characteristic; FA, fatty acid; HNSCC, head and neck squamous cell carcinoma; AUC, area under the curve.

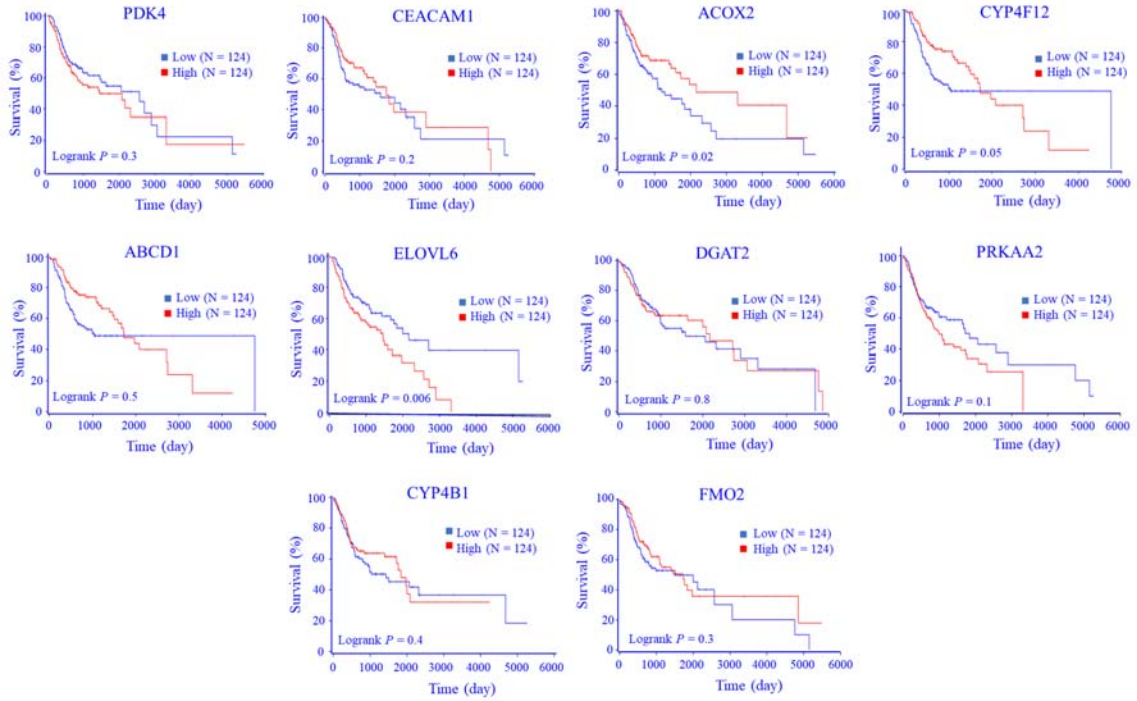

Fig. 7. Cox-regression analysis of top enriched genes from FAM in HNSCC. The prognostic capability of the top enriched genes in FAM was compared using the cox-regression statistical approach available at the OncoLnc database. The low and high expressing groups were shown with blue and red colors, respectively. The log-rank $P < 0.05$ was considered as statistically significant. The ELOVL6 gene demonstrated the greatest prognostic potential in comparison to the rest of the genes in the HNSCC. FAM, Fatty acid metabolism; HNSCC, head and neck squamous cell carcinoma.

DISCUSSION

HNSCC is one of the commonly found neoplasms whose dysregulated metabolic profile remains poorly unknown. Understanding the expression patterns of metabolic pathways in the HNSCC can provide a useful background for the development of future therapeutic approaches that are focused specifically on the metabolic profile of patients with HNSCC (21,42,43).

Lipid metabolism reprogramming in cancer cells is one of the highly investigated areas of the cancer molecular biology field (44,45). FAM is an important part of the metabolic pathways in cancer cells that demands adaptive regulation and changes according to cellular environmental conditions and the growth rate of the fast-dividing cancer cells (16,45-47). However, the exact expression alterations in FA-associated metabolic pathways in the HNSCC were still poorly understood. Therefore, the current study explored the enrichment and expression patterns of molecular pathways associated with the elongation, biosynthesis, catabolism, homeostasis, and metabolism of FAs in the HNSCC tissue samples. With the use of GSEA analysis, fatty acid elongation process was found to be enriched mostly in normal sample phenotypes, while the rest of the pathways, especially the biosynthesis and catabolism of FAs, were notably enriched in HNSCC samples.

The top 10 highly enriched genes in FAM pathways were analyzed with the differential gene expression analysis method in the HNSCC expression dataset, and it was found that all genes except ABCD1 were significantly downregulated in HNSCC. The ABCD1 gene was commonly highly enriched in both FA elongation and FA homeostasis processes. ABCD1 is a protein located in the peroxisome membrane and is responsible for the transfer of FAs into the peroxisome. Previous studies have indicated that the ABCD1 gene was downregulated in human renal cell carcinoma, while its expression pattern in other types of cancers, including HNSCC, is notably unknown (48,49).

In the HNSCC samples, the expression levels of CYP4B1 and FMO2 genes were significantly lower compared to normal samples. The CYP4B1 gene belongs to the cytochrome P450 enzyme family, and its function is associated with the metabolism of xenobiotics (50,51). Many studies have suggested that the CYP4B1 gene could be a possible prognostic biomarker in bladder cancer, lung cancer, and nasopharyngeal carcinoma (49,52,53), and the present study also suggested a great prognostic capability of the CYP4B1 gene in HNSCC as well. FMO2 is a flavin-containing monooxygenase enzyme that belongs to the flavin-containing monooxygenase gene family, which its enzymatic activity is important in the metabolism of drugs, and its expression level has been reported to correlate with the carcinogenesis of oral squamous cell carcinoma, metastatic gastric cancer, breast cancer, and ovarian cancer, and its expression level was also reported to be significantly lower in the HNSCC tissue samples (54-58). Data analysis also confirmed the downregulated expression level of the FMO2 gene in HNSC cancer samples. The results achieved from correlation analysis with the Pearson method among the top enriched genes also revealed that the expression levels of the PDK4, PPKAA2, CYP4B1, CEACAM1, and CYP4F12 genes had moderate correlation, while the expression level of the ELOVL6 gene had a negative correlation with the DGAT2 gene in the cancer tissue samples.

The PPI network analysis of FAM-associated pathways revealed a notable interconnectivity between them and genes including ACOX1, enoyl-CoA hydratase, and 3-hydroxyacyl CoA dehydrogenase (EHHADH), acyl-CoA synthetase long chain family member 1 (ACSL1), PPARA, and FASN, demonstrating the greatest connectivity with the rest of the genes in the network. The GO analysis of the gene sets involved in FAM revealed that most of the genes were involved in molecular functions such as identical protein binding, ATP, and iron ion binding. Iron plays a notable role as a necessary element for multiple enzymes involved in the metabolism of FAs. For instance, fatty acid desaturase enzymes rely on iron to be capable of introducing double bonds into carbon chains, resulting in the desaturation of fatty acids. This desaturation process is necessary for the synthesis of unsaturated FAs, which are important components of cell membranes. In addition, iron also affects lipid metabolism by influencing the activity of enzymes responsible for synthesizing and breaking down FAs, such as acyl-CoA synthetase. This enzyme facilitates the activation of FAs, allowing them to be incorporated into various metabolic pathways (59,60).

The metabolism of arachidonic acid and peroxisome proliferator-activated receptor gamma (PPARγ) signaling was also predicted to involve a great number of genes from FAM gene lists, whose role in carcinogenesis in other forms of cancer has already been investigated by other studies as well (61-66). Other studies have also suggested that the inhibition of arachidonic acid metabolism could be an effective approach for the inhibition of cellular growth in cancer cells (61). It has also been previously suggested that the PPARγ gene could be a potential target for the design of specific chemotherapeutic agents for the treatment of oral cancer and could also be a good candidate for further investigation on the HNSC cancer treatments (67).

ROC test is a common approach used for the prediction of the diagnostic potential of genes of interest for disease phenotypes. This study analyzed the diagnostic potential of the top 10 enriched genes in FAM with the ROC method and exhibited that the diagnostic potential of the selected genes in HNSCC was not significant, except for the CYP4B1 and FMO2 genes. The CYP4B1 and FMO2 genes demonstrated statistically significant AUC values, which suggested that the genes could be suitable diagnostic candidates in the screening and detection of HNSCC. Previous studies have reported a high diagnostic potential for the CYP4B1 gene in bladder cancer and lung adenocarcinoma as well (49,52).

By using the Cox-regression approach, the prognostic capability of the selected genes in HNSCC was assessed and found that ACOX2, CYP4F12, and ELOVL6 genes could be ideal prognostic biomarkers for the estimation of

survival period in patients with HNSCC. Also, other studies have noticed a notable prognostic potential for the ACOX2 gene in hepatocellular carcinoma, breast and lung cancer (68-70). A recent study has also reported that the CYP4F12 gene could be a high-potential biomarker as its overexpression correlated significantly with cellular migration and improved cellular adhesion by the inhibition of the epithelial-mesenchymal transition in HNSC cancer cells (71).

By GSEA analysis, the current study found that the ELOVL6 gene was highly enriched in the fatty acid elongation process and represented a more statistically significant log-rank *P*-value in HNSCC patients compared to other genes involved in FAM. The differential expression analysis also revealed that ACOX2 (Log2 FC = -2.29, *P* = 2.76 E-28), CYP4F12 (Log2 FC = -3.43, *P* = 6.72 E-15), and ELOVL6 (Log2 FC = -1.20, *P* = 1.60 E-10) genes were downregulated in HNSCC samples compared to normal tissue samples. Each cancer has its own expression profile, and biomarkers should be invested in specifically according to the type of cancer cell and cellular origin. However, due to the lack of knowledge and investigations in the prognostic potential of the candidate genes in patients with HNSCC, it would be beneficial to consider that the expression level of the ELOVL6 gene has also been reported by other studies to correlate with poor prognosis in cancer types such as breast, colorectal, and hepatocellular (72-74). Cox-regression analysis revealed that the ELOVL6 gene exhibited the most significant Log-rank P-value (0.006) among the evaluated genes. Patients with HNSCC expressing high levels of ELOVL6 (high-risk group) had significantly shorter survival times compared to those with low ELOVL6 expression (low-risk group), who demonstrated markedly longer survival. Another study also observed that ELOVL6 expression in HNSCC was associated with clinicopathological factors such as grade, survival time, and poor prognosis (75), which was also predicted by Cox-regression analysis in the present study.

Overall, the current study investigated the expression patterns of different gene sets associated with the metabolism of FAs in HNSCC and identified high-potential diagnostic and prognostic biomarker candidates that could be investigated for the design of new therapeutic approaches for the treatment of HNSCC based on the expression profile of FAs in patients. However, this study included several limitations, such as a lack of normal tissue sample counts and cellular investigations on HNSCC cells. Currently, very few studies have investigated the dysregulated patterns of FAM in the HNSCC, and the potential of targeting these pathways for the treatment of head and neck cancer patients is poorly understood (17). The treatment of HNSCC cells with short-chain FAs such as arginine butyrate and α-lipoic acid is effective in the suppression of cellular growth (76).The levels of short-chain FAs in the plasma of HNSC patients that received radiotherapy treatment have also been suggested to be associated with immunometabolic and inflammatory responses (77).

Treatment options for HNSCC may involve a combination of approaches, considering the tumor stage, location, and the patient's overall health. Surgical resection and high-energy radiation are two common methods used to excise the tumor and potentially affected surrounding tissues. It can be a curative option, particularly for early-stage HNSCC. Success rates vary based on the tumor stage and location, but radiation therapy can lead to 5-year survival rates (78,79). Chemotherapy, immunotherapy, and targeted therapy are also useful therapeutic approaches used in the treatment of HNSCC (80-82). Understanding of the molecular biology of HNSCC plays a significant role in the improvement of the sensitivity and success rates of these strategies (82).

Immunotherapy has demonstrated variable success rates in HNSCC, with some patients experiencing prolonged responses. programmed death-1 inhibitors, such as nivolumab and pembrolizumab, have shown notable efficacy in recurrent or metastatic HNSCC (83). Several randomized trials and meta-analyses have shown that induction chemotherapy, typically involving a combination of cisplatin and fluorouracil combined with a local treatment or concurrent chemoradiotherapy (CCRT), resulted in better survival and preservation of organs in patients

with advanced HNSCC, and those who were treated with CCRT using cisplatin tend to exhibit higher survival rates as well (84). Advancements in understanding the underlying biology of HNSCC, the use of biomarker analysis, and targeted treatment strategies have resulted in a notable shift towards individualized therapy in head and neck cancer (85).

Therefore, future studies and investigations are needed to clarify the underlying molecular mechanism and interactions involved in the metabolism of FAs with varying carbon chain length that are dysregulated in the HNSCC and could be targeted for the design of new small-molecule drugs that could alter these pathways and possibly help with the treatment of patients with HNSCC. Future studies are also suggested to investigate and develop prognostic models based on the introduced gene biomarkers and further examine their prediction potential in the overall survival rates of patients with head and neck cancer.

CONCLUSION

HNSCC is one of the most common forms of cancer with a short survival period. The metabolic profile of HNSC cancer was poorly investigated, and the current study reported a dysregulated expression and enrichment pattern of FA-associated pathways in HNSCC. For the diagnosis and prognosis of HNSCC based on the metabolic profile of patients, CYP4B1, FMO2, ACOX2, CYP4F12, and ELOVL6 genes could be potential biomarkers for further investigation.

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Conflict of interest statement

All authors declared no conflict of interest in this study.

Authors' contributions

B. Yazdani, S.A.H. Fesharaki, S.K. Abari, and H. Sirous performed the study design; B. Yazdani analyzed data; B. Yazdani, S.A.H. Fesharaki, S.K. Abari, H. Farajollahi, F.P. Kalashami, A. Zadsar, and H. Sirous performed interpretations of the data and bioinformatics analysis; S.A.H. Fesharaki, S.K. Abari, B. Yazdani, H. Farajollahi, F.P. Kalashami, A. Zadsar, and H. Sirous participated in manuscript writing. All authors read and approved the final version of the manuscript.

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