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International Journal of Molecular and Immuno Oncology

Original Article

mRNA expression of ferroptosis-related proteins in squamous cell carcinoma of tongue

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Received : 07 January 2020
Accepted : 10 February 2020
Published : 13 May 2020

DOI
10.25259/IJMIO_2_2020

Quick Response Code:

ABSTRACT

Objective: Glossal Squamous Cell Carcinoma (GSCC) has a distinct molecular profile including alterations in programed regulated cell death (RCD). Ferroptosis is a newly reported form of RCD mediated by ferric ions and mediated by several proteins. Till date, the possibility of this phenomenon in GSCC has not been explored.

Materials and Methods: The proteins associated with ferroptosis were collated. The mRNA of corresponding proteins expression from GSCC lesional tissues and control tissues from the same patient from the human cancer genome project was obtained as raw values and subjected to differential expression (DE) analysis using DESeq2 statistics. The Benjamini and Hochberg (false discovery rate) approach was used to adjust *P*-value and $P \leq 0.05$ was set at statistical significance. Log fold change was set at 0.6. The significant genes were subjected to network analysis for protein-protein interaction and this network was analyzed for pathway enrichment analysis and presented.

Results: There were 12 cases that had GSCC and controls ($n = 12$). There were 40 genes directly involved with ferroptosis and another 50 genes associated with the ferroptosis. Of this, 28 genes had DE, of which 16 genes were directly involved in the ferroptosis pathway and 12 genes associated with it. These genes, in enrichment analysis, indicated that the other pathways involved several other cancers and cancer signaling. Certain pro-ferroptotic genes and anti-ferroptotic genes had DE. Increase of PTGS2 and ACSL4 mRNA expression in GSCC tissue as compared to control was a significant finding. There is an increase of mRNA of genes that increase the ferric ions intracellularly. These findings would help to draw better and effective treatment strategies that can help to target neoplastic cells in GSCC.

Conclusion: In GSCC, ferroptosis is a possibility and the differential expression of certain pro-ferroptotic genes could he harnessed to draw effective and safer treatment strategies.

Keywords: Tongue cancer, Ferroptosis, Ferritinophagy, Fenton reaction, Glossal cancer

INTRODUCTION

Global incidence of glossal squamous cell carcinoma (GSCC) is on increase, with younger and females being more commonly being affected. Interestingly, in a subset of this patient, the traditional carcinogenic agents such as tobacco and alcohol use were absent. In any case, the morbidity associated with the GSCC and its treatment substantially decreases the quality of life of survivors.[1-3] The molecular mechanisms of the GSCC, particularly of the non-habit associated subset, have been previously described.[4-6]

Of the several molecular mechanisms and pathways, alterations in the classical regulated cell death (RCD) are a key pathway through which genes in GSCC act. Suppression of pro-

apoptotic genes, mutation of the key apoptotic genes, negative regulation of the apoptotic pathway, and evasion of this RCD surveillance are some of the ways by which the RCD is evaded.[6] Besides apoptosis, there are several other RCDs such as forms of necrosis and autophagy. Of these, ferroptosis is a morphologically, biochemically, as well as genetically distinct RCD.[7] It is characteristically an iron-dependent RCD that is initialized by GSH depletion and/or inactivation of GPX4 activity. Besides the GSH-GPX4-mediated ferroptosis, alterations in several other normal cellular process such as Fenton-like reaction of pre-formed lipid hydroperoxides, iron uptake, Fe^{2+} segregation, tricarboxylic acid cycle, and hypoxia-inducible factors, that may contribute to ferroptosis sensitization.[9]

Ferroptosis is characterized by special and distinct changes in mitochondrial morphology such as loss of structural integrity, reduced size, condensed mitochondrial membrane densities, reduced or absent mitochondria crista, and also mitochondrial membrane damage. A subtle form of ferroptosis (ferritinophagy), akin to apoptosis, but mediated by ferric iron, is mediated by nuclear receptor coactivator 4 protein (NCO_4) but once other forms of RCD are established, this is often diminished. There is a constant, overwhelming, iron-dependent accumulation of lipid peroxidation products that ultimately are responsible for the cell death. The detailed molecular mechanism behind ferroptosis has been described.[8-11] This process, being involved with TP53, iron, and metabolic events, if properly harnessed, can form effective GSCC treatment protocols.[11] Early results in this direction indicate that inducing ferroptosis may be a new form of therapeutic anticancer strategy using light-activated molecules to harness photodynamic properties.[12]

The role of autophagy and apoptosis in GSCC has been adequately documented.[4,6] However, there are only very few studies that describe ferroptosis in GSCC.[12] This manuscript attempts to identify the presence of ferroptosis-related transcriptome (RNA) expression in GSCC and compare it to normal control tissue from the same patient. The goal was to study the differential expression (DE) of the ferroptosis-related genes.

MATERIALS AND METHODS

The human genes involved in the biological process of ferroptosis were listed out (https://www.genome.jp/dbget-bin/www_bget?pathway+hsa04216). From recent published literature, the genes that are modified or altered due to ferroptosis were picked out.[7-11] The expression of RNA of the ferroptosis related genes was studied.

The results here are in whole based on data generated by the TCGA Research Network http://cancergenome.nih.gov. The data for this study were from the Human Cancer Genome Atlas at http://portal.gdc.cancer.gov/. Only those tongue cancer (except the base of tongue) that had normal tissues from the same patients as control were included for this study. The raw transcriptome profiling of lesional and control tissue RNA sequencing was collected from the web portal.

After the initial sample collection and processing as detailed in the previous publication, the first step was to study the RNA-Seq alignment.[13] The count of the "reads" mapped to each gene. Then, the counts were performed using a Python-based program – (high-throughput) $HTSeq^2$ and are calculated at the gene level. All such individual genes, using Ensembl gene names or HUGO gene symbol, were listed and collected as.txt files and processed.[14]

The data were then subjected to DE analysis using the www.networkanalysts.ca.[15] In the portal, the single gene expression table mode was used. The variance filter (to remove data that are less informative or erroneous) was set at the 15th percentile and low abundance to filter off the data with count lower than a threshold set at minimum. Data quality was checked with principal component analysis and data normalization (for reliable detection of mRNA transcriptional differences and ensure uniform count distribution) was done using Log_2 counts per million. DE analysis was performed DESeq2 statistics.[16]

To identify the difference between GSCC lesional and adjacent normal tissue, in terms of the ferroptosis genes studied, the adjusted *P*-value was set as ≤0.05, the data presented along with log fold change (LFC), LFC standard error, and Wald's statistics. LFC threshold was preserved at 0.6, and actual obtained values quoted along with *P*-value, as there are contentions with regard to LFC. LFC is a measure of change in the expression level of genes. It inherently carries a disadvantage of being biased and misclassified. It would miss the DE entities with large differences but smaller ratios, leading to inaccurate identification of changes, especially high expression levels.[17]

The significant genes were subjected to protein-protein interaction (PPI) network analysis using InnateDB as reference.[18] If the network was large, an attempt was also made to minimize the network, to identify important nodes, edges, and seeds. The former network was subjected to KEGG pathway enrichment analysis (upregulated, downregulated, and all) and presented.[19]

RESULTS

Of the 530, head-and-neck cancers, listed with the HNSC database, there were 12 GSCC, all with non-specified, non-base of tongue site having RNA counts of lesional and normal control tissue. This sample formed the basis of this study. The mean age of the study population was 61 ± 14.6 years (range: 32–87 years), with 0.23 ± 0.11 fraction genome altered,

average mutation count 127.08 ± 126.69 (range: 43–506), and an aneuploidy score 11.42 ± 7.93. Of the 12 cases, 6 were males, 6 had been non-smokers, 2 had no TP53 mutation, 6 in ICD-10.3 clinical Stage-II, 2 in Stage-III, and 4 in Stage IVA. Histologically, 9 cases were in G2 Grade and 3 in G3.

There were 40 genes in ferroptosis as per the KEGG pathway and another 50 genes identified to be associated with ferroptosis from literature. There were 60,483 genes in each of the tissue studied, at HNSC database. The 90 ferroptosis-related genes were identified from this database and subjected to DE as outlined above. We checked for the difference in the crucial GPX4 expression in GSCC tissues and controls. The difference of GPX4 between cases and controls and that of the wildTP53 and mutated TP53 were not statistically significant (P = 0.295 and 0.288, respectively). Among the controls, all were wild TP53, with a mean GPX4 of 7319.42 ± 3375.53 while in GSCC, the mean GPX4 in wild TP3 expression cases was 8151.5 ± 2826.31 while those with mutated TP53 had 9140.00 ± 4464.85.

In the DE analysis, due to low abundance and variation, only 69 genes were processed. PCA diagram is shown in Figure 1a and b. Of these 69 genes, there were 28 genes that were DE had logFC of 0.6 and adjusted $P \leq 0.05$. The PCA and heat map diagram of these genes are shown in Figure 1a and b. The ferroptosis pathway genes that were DE with statistical significance are shown in the KEGG pathway diagram [Figure 2].

Of this, 16 were related to KEGG pathway and 12 related to be associated with ferroptosis [Table 1]. These 28 genes were subjected to PPI network analysis using InnateDB. There was no single central continent and small linked island chains were formed with surrounding networks created by MAP1LC3C, SLC3A2, PTGS2, PPARGC1A, SLC2A4, TF, FTH1, PEBP1, CYBB, CP, FTL, ITGB4, and HSPB1. In all, there were 502 nodes (related proteins), forming 578 edges (interconnecting interaction) from the 20 seed genes [Figure 3a]. The minimized network was made of 49 nodes, 87 edges from the 20 seeds [Figure 3b]. The KEGG pathway enrichment analysis showed the involvement of 5 upregulated and 6 downregulated ferroptotic genes with high statistical significance. The overall node mode revealed that several cancer-related pathway being involved with high statistical significance. The 25 such top-most cancer-related pathways involved are being depicted in Table 2.

DISCUSSION

Ferroptosis was first described in 2012 and since then has been notably associated with several cancers.[7-11] The molecular basis of the ferroptosis has been not fully deciphered. There have been scores of genes associated with ferroptosis. Increased gene expression (as mRNA) of CHAC1, PTGS2, and protein of ACSL4 could serve as a marker of ferroptosis under several conditions.[20] Till date, the existence of this phenomenon and genetic expression of ferroptosis-related genes in GSCC have not been studied and this study, probably, is the first to specifically attempt address the lacunae using existing patient's previous RNA level data, though cell line level studies have been reported.

In this study, the increase of PTGS2 and ACSL4 mRNA expression in GSCC tissue as compared to control was a significant finding. This could possibly indicate that ferroptosis is associated with GSCC. In the present study, we observed that 15 ferroptosis genes were in the KEGG pathway that was differentially expressed with significance (adjusted $P \leq 0.05$, LogFC=0.6). Of this ACSL4, FTL, PRNP, SLC9A14, SLC3A2, STEAP3, and TFRC (TFR1) were increased in GSCC while the ALOX15, CP, MAP1LC3C, SAT2, SLC11A2, TF, TP53, and VDAC3 were decreased in GSCC.

The SLC3A2, a cell membrane-based solute carrier protein's mRNA, was increased in GSCC. This is an important part

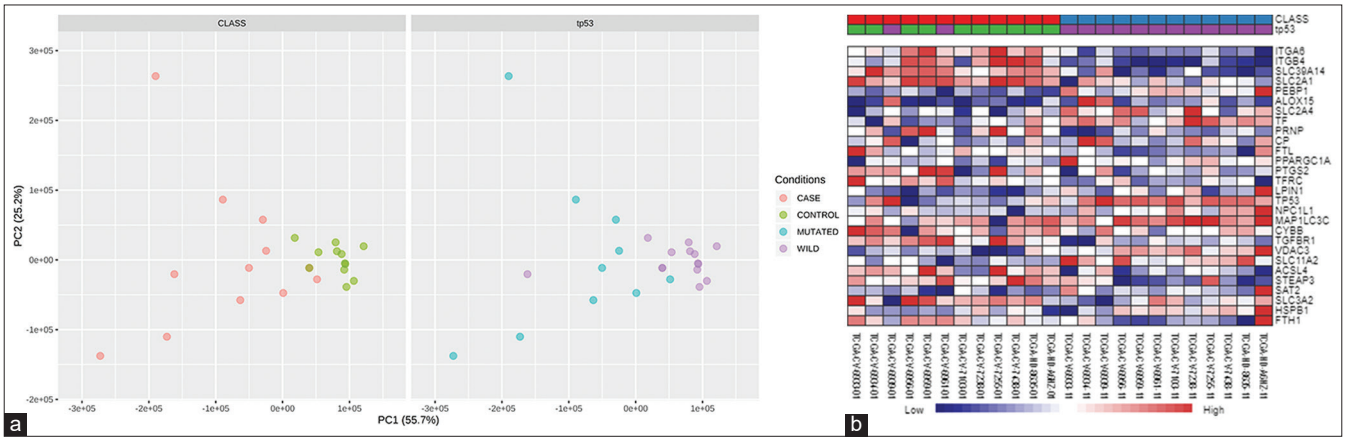

Figure 1: (a) The Principal Component Analysis of the mRNA expressions classified by their status (case/control) and TP53 mutation, (b) Heat map showing the expression of the mRNA across the study group.

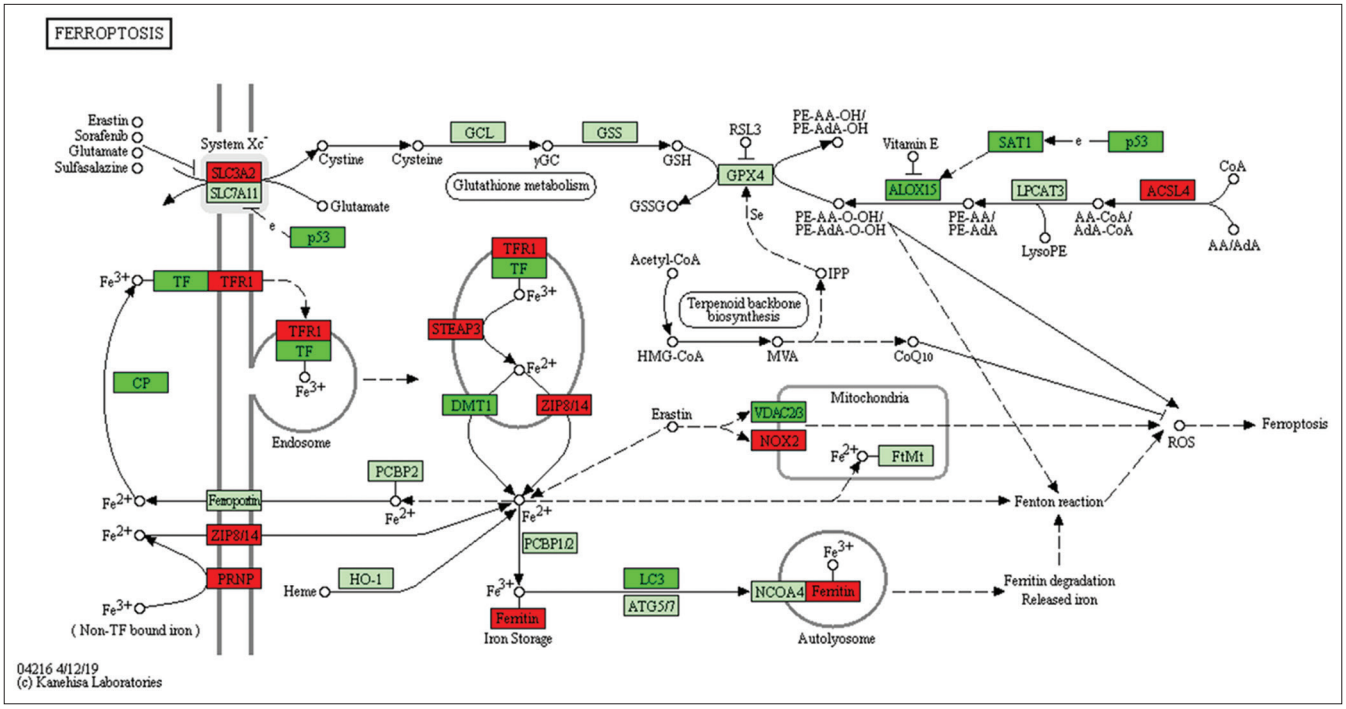

Figure 2: Ferroptosis pathway as modified from KEGG pathway. The red color indicates the upregulated mRNA in this study and bright green the downregulated mRNA as observed in this study.

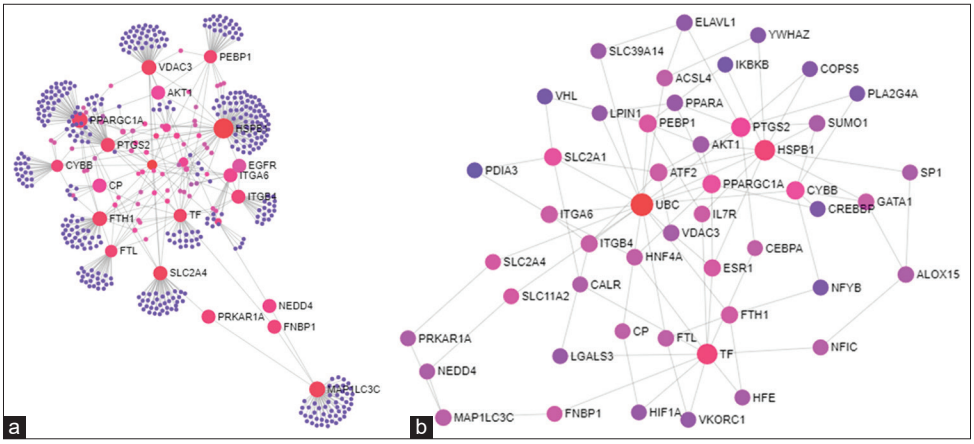

Figure 3: (a) The protein-protein interaction network analysis of the significant deferentially expressed genes in this study, (b) The minimized protein-protein interaction network analysis of the significant differentially expressed genes in this study.

of the system XC that mediates cysteine transport into cells. System XC is a heterodimer composed of SLC7A11 and SLC3A2 found in cell membrane. It is a sodium ion independent cystine/glutamate transporter that can mediate transfer cystine into the cells and export glutamic acid out of the cells using energy. Suppression of the system leads to decreased uptake of cysteine (oxidized form of cysteine). Cysteine, in normal cells, is acted on by GSH synthase to convert GSH to GSSGO. The decrease of cystine would lead to the decrease of cysteine and thereby exhaustion of GSH. GSH serves as a cofactor of GPX4. GPX4 reduces the lipid peroxidation that causes lipid peroxides that ultimately kill the cells.[7-11]

On a single gene analysis, we identified reduced GPX4 in the GSCC with wild TP53 and increased GPX4 in mutated TP53. However, GPX4 was not DE between the GSCC and control while ACSL4 was DE. The formation of lipid peroxide and 15-hydroperoxy arachidonoyl-phosphatidylethanolamine is initiated by the enzyme coded by ACSL4, the increased expression of which is a marker of ferroptosis.[8-11,20] ACSL4 is also possibly modulated through the Src by the α6β4 integrin.[21] As the α6β4 integrin is highly upregulated in

Table 1: Significantly different genes between glossal squamous cell carcinoma and normal controls.

Genes	LogFC	Base mean	LFC SE	Stat	*P*-value	Adjusted *P*-value
Ferroptosis pathway associated genes						
SLC39A14	1.6475	4277.8	0.27933	5.8981	3.68E-09	7.85E-08
ALOX15	−3.1586	196.53	0.66751	-4.7318	2.23E-06	2.33E-05
TF	−2.6713	2170.1	0.61011	-4.3784	1.20E-05	8.50E-05
PRNP	1.1979	18,362	0.31028	3.8609	0.000113	0.000565
CP	−2.3703	1838.4	0.61456	-3.8569	0.000115	0.000565
FTL	1.0496	65,129	0.28645	3.664	0.000248	0.001135
TFRC	1.3111	11,347	0.38786	3.3804	0.000724	0.002574
MAP1LC3C	−1.2562	22.012	0.46537	-2.6993	0.006949	0.017788
CYBB	1.0626	1687.4	0.46614	2.2797	0.022628	0.046715
VDAC3	−0.64952	4600.8	0.15866	−4.0937	4.24E-05	0.000272
SLC11A2	−0.89975	3434.2	0.22815	−3.9437	8.02E-05	0.000467
ACSL4	0.85454	4521.3	0.23636	3.6154	0.0003	0.001267
STEAP3	0.71362	3785.3	0.24315	2.9349	0.003337	0.009707
SAT2	−0.87571	1472.3	0.31935	-2.7421	0.006104	0.016986
SLC3A2	0.66757	16144	0.27222	2.4523	0.014196	0.033649
FTH1	0.79344	23,559	0.33659	2.3573	0.018409	0.039273
Genes associated with ferroptosis						
ITGA6	2.2824	47,776	0.33971	6.7187	1.83E-11	1.17E-09
ITGB4	1.7353	49,688	0.27987	6.2005	5.63E-10	1.80E-08
SLC2A1	1.9491	48,466	0.35425	5.5021	3.75E-08	6.00E-07
PEBP1	−1.1167	13,659	0.23417	−4.7687	1.85E-06	2.33E-05
SLC2A4	−2.3832	691.84	0.50656	−4.7046	2.54E-06	2.33E-05
PPARGC1A	−1.4047	389.86	0.39037	−3.5984	0.00032	0.001267
PTGS2	1.8421	2276.4	0.51377	3.5855	0.000337	0.001267
LPIN1	−1.0931	2005.5	0.32658	−3.3472	0.000816	0.00275
TP53	−1.1204	3132.6	0.33785	−3.3161	0.000913	0.002921
NPC1L1	−1.3729	17.945	0.4211	−3.2604	0.001113	0.003391
TGFBR1	0.92121	4205.2	0.2089	4.4099	1.03E-05	8.27E-05
HSPB1	−0.90944	97,762	0.37683	−2.4134	0.015804	0.036124

Log FC: Log of fold change, LFC SE: Log fold change standard error, Stat: T statistics

GSCC, there could be a Src-mediated repression of ACSL4. ACSL4 increases the pro-ferroptotic lipid composition along with the plasma membrane. Extracellular matrix (ECM) detachment is reported to enhance ferroptosis and apoptosis, through anoikis, intracellular reactive oxygen species (ROS), causing cell death. In GSCC, possibly, the ECM-detached cells could have adapted to trigger an increase of α6β4 integrin as a mechanism to prevent both ferroptosis and apoptosis.[21] This finding of the increased ACSL4 and increased α6β4 level relationship in GSCC, though contradictory, has to be investigated further.

ALOX15, a gene whose protein is involved in conversion of arachidonoyl-phosphatidylethanolamine to 15-hydroperoxy arachidonoyl-phosphatidylethanolamine, is reduced in GSCC. This lipid peroxide is acted on by the GPX4, which when suppressed leads to ferroptosis. Decrease of ALOX15, as seen in GSCC, could cause less arachidonoyl-

Table 2: KEGG enrichment analysis of the protein-protein interaction network developed from significant genes that were differentially expressed between glossal squamous cell carcinoma and controls for ferroptosis.

Pathway	Total	Expected	Hits	*P*-value	False detection rate
Upregulated nodes					
Ferroptosis	40	0.0465	5	3.54E-10	1.13E-07
Downregulated nodes					
Ferroptosis	40	0.0517	6	2.67E-12	8.49E-10
All nodes					
Pathways in cancer	530	23.2	74	3.71E-20	5.90E-18
Autophagy–animal	128	5.61	33	3.23E-17	3.42E-15
Ferroptosis	40	1.75	18	1.07E-14	5.66E-13
MAPK signaling pathway	295	12.9	45	8.60E-14	3.42E-12
Focal adhesion	199	8.72	36	1.72E-13	6.08E-12
HIF-1 signaling pathway	100	4.38	24	4.28E-12	9.08E-11
Cellular senescence	160	7.01	30	7.81E-12	1.46E-10
Autophagy–other	32	1.4	14	1.70E-11	2.84E-10
Longevity regulating pathway	89	3.9	21	1.38E-10	2.09E-09
TNF signaling pathway	110	4.82	23	2.45E-10	3.11E-09
Toll-like receptor signaling pathway	104	4.56	22	4.80E-10	5.65E-09
PI3K-Akt signaling pathway	354	15.5	42	2.32E-09	2.23E-08
ErbB signaling pathway	85	3.72	18	1.86E-08	1.29E-07
Apoptosis	136	5.96	23	1.87E-08	1.29E-07
Adipocytokine signaling pathway	69	3.02	16	2.98E-08	2.02E-07
Viral carcinogenesis	201	8.81	28	4.31E-08	2.80E-07
Proteoglycans in cancer	201	8.81	28	4.31E-08	2.80E-07
Choline metabolism in cancer	99	4.34	17	1.12E-06	6.11E-06
VEGF signaling pathway	59	2.59	13	1.13E-06	6.11E-06
Cell cycle	124	5.43	19	1.60E-06	8.46E-06
Regulation of actin cytoskeleton	214	9.38	26	1.99E-06	1.04E-05
Estrogen signaling pathway	138	6.05	20	2.08E-06	1.07E-05
Adherens junction	72	3.15	14	2.18E-06	1.10E-05
mTOR signaling pathway	153	6.7	21	2.83E-06	1.41E-05
PPAR signaling pathway	74	3.24	14	3.07E-06	1.50E-05

phosphatidylethanolamine formation, thereby reducing ferroptosis. On the other hand, reduction of ALOX15 levels has been associated with certain cancers.[22,23] Furthermore, SAT2, a molecule associated with ALOX15 in lipid peroxide metabolism, is downregulated in GSCC.[24] These results indicate that possibly the ferroptosis mechanism was initiated but slowed down later through the GPX4 pathway.

PRNP is found in cell membrane that is associated with change of ferric to ferrous ion and its transfer into cell in a non-TF bound form.[7-11,25] The expression is increased in GSCC. FTL and FTH1 are proteins concerned with iron sequestration, are increased in GSCC. TF and TFRC are found in cell wall, mitochondria, and endosomes. They are associated with iron transport in the cell.[7-11,25] In GSCC, FTL, FTH1, and TFRC are increased. Most of the iron is transported into cells by TF-dependent manner, using TFRC into the cell as endosome. In the endosome, TFRC-TF complex, together with STEAP3 and SLC39A14 (ZIP-14), leads to increase of ferric ions inside the cells. The mRNA of STEAP3 and SLC39A14 is increased in GSCC. The accumulation of excess of ferric ion through

the Fenton reaction could lead to reactive oxygen species (ROS) formation that would kill cells.[25] SLC39A14, that is, upregulated in GSCC, in cell membrane, is associated with intracellular transfer of ferric ions.[25] TF, an essential part of the TF-TFRC complex, is downregulated in GSCC, while TFRC in upregulated. Iron regulators such ceruloplasmin (CR, mediates extracellular ferric to ferrous form) are also downregulated. The endosome-bound SLC11A2 (DMT1) is downregulated in GSCC. The ferric form of iron transporter regulators is upregulated in GSCC, indicating that there is an accumulation of the ferric ions intracellularly, which could trigger Fenton reaction, leading to ferroptosis or sensitization to ferroptosis.[7-11,25]

Mitochondria membrane-based voltage-dependent anion channel (VDAC3) was downregulated while CYBB (NOX2) was upregulated in GSCC. Mitochondria membrane genes that could turn pro-ferroptotic in the presence of erastin [Figure 1b and 2]. This could lead to increase the free ferric ion intracellularly and predisposing to Fenton route of ferroptosis. Similarly, MAP1L3C3, a marker of autophagy, has been reduced in GSCC. This when considered together with the fact that NCOA4, ATG5, ATG7, PCBP1, and PCBP2 are not differentially expressed between GSCC and normal tissue, could possibly indicate that the ferritinophagy is absent in the GSCC.[26-29]

TP53 has dual role in ferroptosis. It can promote as well as inhibit the process.[30-32] It modulates SAT2, which is downregulated in GSCC in the present study. The TP53 mutation appears to confer the cell with increased labile ferric iron pool in the cytoplasm, which can trigger ferroptosis in GSCC cells. From the study sample, the possibility of ferritinophagy and GPX4-mediated ferroptosis is at minimum. The preponderance of expression of pro-ferroptotic RNA of ACSL4, TFR1, SLC3A2, STEAP3, SLC39A14 (ZIP14), and PRNP and reduction of pro-ferroptotic SAT2, ALOX15, CP, and TF could be used to create newer pharmacological modalities to trigger ferroptosis. Most of these are TP53 mutation dependent and can be effectively modulated.

HSPB1 is a known negative modulator of ferroptosis, which, in the present study, was also downregulated in the GSCC.[26] In the associated genes, the mRNA of TGFBR1 (ALK5) which is a positive modulator of ferroptosis was increased in GSCC. This is associated with carcinogenic process.[33] SLC2A1 (Glut1) and SLC2A4 (Glut4) were DE between the GSCC and control. The former was upregulated in GSCC while the later was downregulated. By their interaction with TP53 and metabolism, they possibly have a dual role in ferroptosis.[30]

Similarly, diet-related NPC1L1 and adipocyte-related LPIN1 expression, both which are modulated by TP53 were downregulated in GSCC, possibly correlating to the TP53 mRNA expression.[30] PPARGC1A is a transcriptional coactivator that regulates key mitochondrial genes and endosomes.[34] They also play a role in metabolic reprogramming of glucose and fatty acid metabolism. This could possibly interact with TP53 and is downregulated in the GSCC. The scaffolding protein PEBP1 regulates ferroptotic cell death by binding with lipoxygenases and allowing them to generate lipid peroxides.[35] Reduced PEBP1 mRNA expression, in GSCC, could possibly be reflective of reduced ferroptosis.

The mean levels of GPX4 varied between cases and controls and TP53 mutation status appears to play a role in it, though these were not statistically significant. However, the trend possibly reflects that in wild TP53 cases, probably, the GPX 4 is relatively reduced and in mutated TP53 GSCC, it is increased. The role of TP53 in modulating ALOX 15 through the SAT1 and SAT2 and modulating SLC7A11 needs to be explored.

From these foregoing discussions, it is evident that ferritinophagy as autolysosome and GPX4 mediate ferroptosis, though initiated by high expression of pro-ferroptotic ACSL4 and ferritin, does not proceed to cell death, probably due to decreased expression of ALOX15 as well as the absence of NCOA4 expression. Probably, mutation of TP53 and upregulation of α6β4 integrins could have contributed to this phenomenon. On the other hand, increased expression of SLC39A14 (ZIP14) and STEAP3 contributes to increased cytoplasmic pool of ferric ion that by Fenton reaction causes ROS-mediated ferroptosis sensitization. Dysregulated iron metabolism has been previously reported in cancer tissues. Polyunsaturated fatty acid-containing phospholipid hydroperoxides, if not converted to alcohols by GPX4 precipitates ferroptosis. Increased ferric ion, stored intracellularly as ferritin, if crosses the threshold, can end in ferroptosis by enhancing the conversion of polyunsaturated fatty acid-containing phospholipid into polyunsaturated fatty acid-containing phospholipid hydroperoxides by Fenton chemistry and/or by stimulation of ALOX15-ferroptosis pathway.[25] In this study, we noted increase of mRNA of genes that increased free ferric ion in cytoplasm while ALOX15 is suppressed.

The increased ferric ion may sensitize the wild TP53 containing GSCC, potentially leading to ferroptosis. Among mutated TP53 GSCC cases as compared to control wild TP53 group, as certain pro-ferroptotic protein, mRNA levels are increased while others are decreased, it could be inferred that ferroptosis could be diminished. However, such alterations provide newer opportunities to trigger cell death in GSCC, paving the path for newer, safer, targeted treatment modalities. Also, to be noted is the activation of counter mechanism. For example, when pro-ferroptotic TFR1 is increased, TF decreases. If STEAP-3 and SLC39A14 (ZIP14) are increased to counteract, SLC11A2 (DMT1) is

decreased. In mitochondria, increase of VDAC3 appears to be compensated by FTH1 (NOX2) decrease. This compensatory mechanism spreads in GPX4 pathway, endosomes, mitochondria, and cell membrane. This could be effectively used to formulate newer, non-invasive, targeted therapies. Preliminary success of such therapies in cell lines have been reported.[12]

This present study being a secondary data analysis, should be interpreted and extrapolated with caution. The small sample size, non-consideration of mutations, downstream events besides other clinical and genetic factors. In spite of the same, the statistical observations of certain mRNAs being upregulated and others downregulated as compared to control tissues constantly indicate the expression of pro-ferroptotic and anti-ferroptotic genes that are at play in GSCC. The actual DE could be harnessed to device novel treatment strategies.

CONCLUSION

Alteration in physiologic cell death is a key element in malignant transformation as well as progression of carcinogenesis. Certain pro-ferroptotic and anti-ferroptotic genes are differentially expressed in GSCC. These genes are associated with several vital biological processes including cell division, iron transport, cell division, cell cycle, and cell adhesions. Abnormalities in the mRNA expression of these proteins have an impact on several cancer-related pathways. This DE could be effectively harnessed to develop novel strategies to conquer GSCC.

Declaration of patient consent

Patient's consent not required as patients identity is not disclosed or compromised.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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How to cite this article: Thavarajah R. mRNA expression of ferroptosis-related proteins in squamous cell carcinoma of tongue. Int J Mol Immuno Oncol. 2020;5(2):73-81.