Deregulated Expression of SIRT3/FOXO3a/SOD2 Axis is Associated with Poor Survival in HNC Patients

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ABSTRACT

Current study was conducted to assess the expression variations of UPR^{mt} genes such as SIRT3, FOXO3a and SOD2 in head and neck cancer (HNC). In present study 200 HNC tumors and adjacent uninvolved sections, taken as controls, were used. q-RT PCR was performed for expression analysis of selected genes in HNC. Data analysis showed significant down-regulation of SIRT3 ($p< 0.0001$) and significant up-regulation of FOXO3a ($p< 0.0001$) and SOD2 ($p< 0.0001$) in HNC tumors compared to control sections. Expression levels of selected genes were correlated using spearman correlation and SIRT3 showed significant negative association with SOD2 ($r= -0.372$; $p< 0.02$) and FOXO3a ($r= -0.669$; $p< 0.01$). A significant positive association was observed between the FOXO3a with SOD2 ($r= 0.447^{**}$; $p< 0.01$) and SIRT3 with Ki-67 ($r= 0.391$; $p< 0.02$) in HNC patients. Kaplan-Meier analysis showed down regulation of SIRT3 (logrank p= 0.02, HR= 1.90, 95% CI= 1.63-2.21) and up-regulation of FOXO3a (logrank p= 0.03, HR= 1.88, 95% CI= 1.41-2.49) and SOD2 (logrank p= 0.0009, HR= 2.54, 95% CI= 1.31-4.90) was observed associated with decreased survival of HNC patients. These data suggest that deregulated expression of UPR^{mt} genes act as the independent prognostic markers in HNC.

Keywords: UPRmt genes, SIRT3, FOXO3a, SOD2, HNC

INTRODUCTION

Head & neck cancer (HNC) constitutes 8-10% of all cancers in southern Asia. The actual burden of HNC in Pakistan has remained 18.74% of all new cases recorded during 2004-2014, but it is increasing significantly due to a multitude of factors. However, due to lack of presence of any proper cancer registry the exact incidence and prevalence figures are not available.[1] Major risk factors of HNC are smoking, alcohol consumption, genetic instability and DNA damage.[2] This DNA damage is produced by increased level of oxidative stress and reactive oxygen species (ROS) in HNC tissue. The production of these ROS is higher in mitochondria compared to nucleus because of its closeness to ROS production center and reduced repair mechanisms.[3] Increased ROS and oxidative damage is responsible for misfolding and protein accumulation in mitochondria and activates mitochondrial un-folded protein response (UPR^{mt}) pathway.

The purpose of UPRmt pathway is to reduce the level of stress due to misfolded proteins by increasing activity of mitochondrial proteases.[4] UPR^{mt} pathway proceed in three axes such as the canonical UPR^{mt} axis, SIRT3 axis and UPRIMS–ERα axis.

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The canonical UPR^{mt} leads to altered levels of transcription factors such as CHOP, ATF4, ATF5 and increases the protein folding capacity inside mitochondria.[5] In UPRIMS–ERα axis, estrogen receptor alpha (ERα) is the key player which is facilitated by AKT via ERα phosphorylation mediated by ROS. Activation of ERα is responsible for the increased transcripts of nuclear respiratory factor 1 (NRF1) and regulates the proteasome level.[5,6] First two axes of UPR^{mt} pathway maintain the protein folding capacity and proteasome level of mitochondria.[6] The third axis of UPR^{mt} pathway (SIRT3 axis) counteracts with proteotoxic oxidative environment of mitochondria and exerts the antioxidant activity. Mitochondrial health is secured by antioxidant activity performed by UPR^{mt} sirtuin axis by neutralize the production of ROS, as a mitochondrial dysfunction byproduct.[7]

UPRmt SIRT3 axis is the main focus of this study and it includes three key players such as NAD-dependent deacetylase Sirtuin 3 (SIRT3), Fork-head box O3 (FOXO3a) and Super-oxide dis-mutase 2 (SOD2). Among these molecules, the SIRT3 is the main coordinator of UPR^{mt} which regulates FOXO3a and SOD2 activities in UPR^{mt} pathway.[5] SIRT3, also known as mitochondrial Sirtuin-3, is a member of sirtuin family, which is a class III NAD dependent deacetylase. In addition to histone deacetylation, SIRT3 is also involved in deacetylation of various metabolic enzymes (like SOD2) and transcription factors.[8] In mitochondria it acts as a negative regulator of ROS production by stimulating effective electron movement via electron transport chain.[9] Increased ROS level can cause oxidative stress, which can change deacetylase function of SIRT3 to ribosyl transferase that contributes towards post translational modification.[10] Activities of SIRT3 have been assessed in carcinogenesis process and expression deregulation of SIRT3 has been reported in several malignancies such as breast cancer, gastric cancer, lung cancer and head & neck cancer.[11,12]

FOXO3a, a fork-head transcription factor is an established tumor suppressor gene, its activity depends upon its sub nuclear localization which is achieved through post translational modifications.[13] FOXO3a regulates the expression of many genes which modulate the cellular response to oxidative stress. Alterations in functions of FOXO3a may result in increased oxidative stress.[14] FOXO3a plays a critical role against oxidative stress, and under oxidative stress it acts as a tumor suppressor.[15]

SOD2 is a member of antioxidant enzymes that work to reduce ROS. It is the only enzyme that is important for survival in anaerobic environment under stressful physiological conditions.[16] The strategic SOD2 location in the mitochondria may be linked to its critical role for survival of life. SOD2 has been found mutated in some of the types of human tumors. The relationship of 6q deletion with the SOD2 function has proposed that it may be another sort of tumor suppressor gene.[17] Expression deregulation of SOD2 has been reported in cancer cells compared to its normal counterparts, including lung and esophageal, gastric, and colorectal cancer cells.[18]

Expression analysis of SIRT3,[19] FOXO3a[20] and SOD2[21] has been assessed singly in different cancers. However, no study has been published with respect to expression deregulation of SIRT3/FOXO3a/SOD2 axis of UPRmt pathway. The objective of present study was to measure the mRNA levels of SIRT3, FOXO3a and SOD2 in head & neck cancer patients. Furthermore, gene expression levels were correlated with different histopathological parameters.

PATIENTS and METHODS

Sample and Control Selection

This case control study included 200 head & neck cancer tissue samples and adjacent un-involved, non-cancerous, 2 cm away from tumor sections, microscopically confirmed tissues were used as control samples. Demographic details of study cohort are given in Table 1. The tissue samples / controls were collected from Pakistan Institute of Medical Sciences during 2010-2013. Informed consent was obtained from all participating individuals. All designed experiments followed the principles outlined in Helsinki Declaration.

The inclusion criteria for the patients was;

(i) no prehistory of cancer or any kind of lesion

Table 1. Demographic parameters of Head & Neck cancer patients

Parameters		Patients
Age	≥ 40	140 (70)
	≤ 40	60 (30)
Gender	Males	102 (51)
	Females	98 (49)
Tumor Area	Larynx	60 (30)
	Pharynx	30 (15)
	Nasal Cavity	06 (03)
	Oral Cavity	104 (52)
Tumor Grade	Poorly Differentiated	46 (23)
	Moderately Differentiated	40 (20)
	Well Differentiated	114 (57)
T Stage	T1-T2	130 (65)
	T3-T4	70 (35)
	N Stage	
	N0	102 (51)
	N1-N3	98 (49)
	M Stage	
	M0	186 (93)
	M1	14 (07)

(ii) no history of any other disease with hereditary component like hypertension, cardiac impairment and diabetes (type 2 diabetes).

(iii) histo-pathological confirmed squamous cell carcinoma of head and neck region was included in the present study. Each tumor sample contained at least 94% cancerous cells/tissue.

The surgically excised tissue consisted of tumor and healthy section (to be used as control) which was stored in RNA later solution at –80°C. Cyrosectioning was performed to get thin slices of tissues for hematoxylin and eosin (H/E) staining. Identification of tumor cells and adjacent normal cells in sliced tissues was confirmed by examination of tissue from histopathologist. The controls were confirmed uninvolved by consultant pathologist. Histopathological reports for each sample was obtained and additional information of tumor clinical factors was also obtained. Demographic information about ethnicity, addiction (smoking), age and gender was recorded and used for association with the disease.

Prior ethical approval from ethical review committee of COMSATS University Islamabad was obtained to conduct this study.

RNA Purification & cDNA Synthesis

RNA was extracted from tumor as well as control tissues using Trizol method with slight modifications.[22] Quality of RNA was checked by 1% TAE agarose gel electrophoresis. Spectrophotometry was used to check the quantity of RNA. Sample having 50 ng of RNA or more were processed for cDNA synthesis.

Reverse transcriptase PCR was used to synthesize cDNA from isolated RNA by commercially available kit from Thermo Scientific (USA). Synthesized cDNA was confirmed by β-actin reverse transcriptase PCR.

Expression Analysis

Primer quest tool of IDT Technologies was used to design gene specific primers for SIRT3, FOXO3a, SOD2, and an internal control β-actin. Quantitative real time polymerase chain reaction (q-RT PCR) was used for expression analysis using Step one Plus Thermal cycler (Applied Biosystems). Reaction was performed in triplicate for selected genes and β-actin was used as reference. Each reaction constituted of 10μl SYBR green, 2μl primers, 1μl cDNA and 7μl PCR water. Cyclic conditions included, 5 min of initial denaturation, 1 min of annealing for 40 cycles. Cycle threshold values were taken for each samples and 2-delta delta Ct method was used to calculate relative expression.

Data Analysis

Statistical tools from SPSS and GraphPad Prism were used to analyze the data. SIRT3, FOXO3a

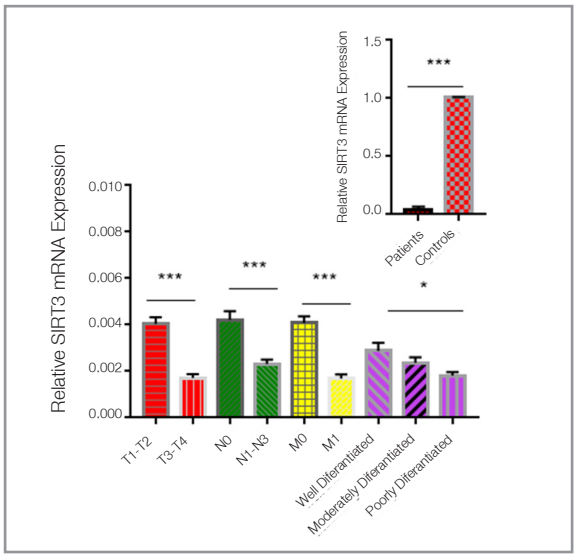

Figure 1. mRNA expression of SIRT3 in HNC tumor samples and normal control samples. SIRT3 mRNA expression in HNC tumor samples with different T-stage, N-stage, M-stage and grades. p< 0.05, ** p< 0.01, *** p< 0.001

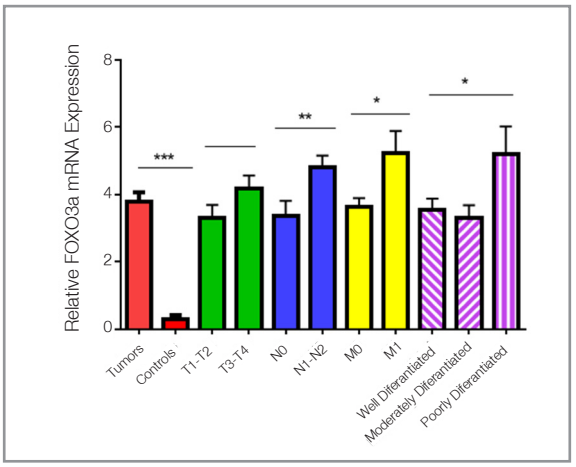

Figure 2. mRNA expression of FOXO3a in HNC tumor samples and normal control samples. FOXO3a mRNA expression in HNC tumor samples with different T-stage, N-stage, M-stage and grades. p< 0.05, ** p< 0.01, *** p< 0.001

and SOD2 expressional values were compared on the basis of demographic characteristics by applying chi square, one-way ANOVA and student t-test. Gene to gene and gene to different histopathological parameters were compared by applying spearman correlation.

Kaplan-Meier and Cox regression analysis for univariate and multivariate was used to correlate the expression level of selected genes with survival status of HNC patients and to measure the prognostic significance of these molecules in HNC. Diagnostic value of SIRT3, FOXO3a and SOD2 genes was assessed using ROC curve analysis.

RESULTS

SIRT3, FOXO3a and SOD2 mRNA levels were measured in 200 head & neck cancer tissue samples and adjacent un-involved control samples using qRT-PCR. SIRT3 mRNA level was significantly reduced in tumors samples compared to control samples (p< 0.00002). SIRT3 expression was found significantly down regulated in advanced T stages (T3-T4; p< 0.00004), N stages (N1-N3; p< 0.0002) and M stage (M1; p< 0.000009) when compared to early T stages (T1-T2), N stages (N0) and M stage (M0 respectively) of HNC patients. In case of tumor grade, SIRT3 was observed significantly down regulated in poorly differentiated samples when compared with well and moderately differentiated samples (p< 0.03) as shown in Figure 1.

FOXO3a mRNA level was significantly increased in tumor samples compared to control samples (p< 0.00003). FOXO3a noticed expression was noted up regulated in advanced N-stage (N1-N3; p< 0.009) and M stages (M1; p< 0.02) as compared to early N stage (N0) and M stages (M0). In case of tumor grade, FOXO3a was observed significantly up regulated in poorly differentiated samples (p< 0.03) compared to well and moderately differentiated tumors as shown in Figure 2

SOD2 mRNA level was detected significantly increased in tumor samples compared to control samples (p< 0.04). SOD2 expression was up regulated in advanced T-stages (T3-T4; p< 0.03), N stages (N1-N3; p < 0.04) and M stages (M1; p< 0.007) compared to early T-stages (T1-T2), N-stages (N0) and M-stages (M0). In case of tumor grade, SOD2 was observed significantly up regulated in poorly

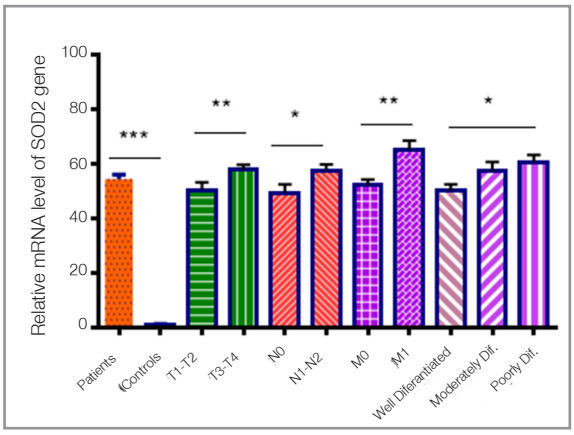

Figure 3. mRNA expression of SOD2 in HNC tumor samples and normal control samples. SOD2 mRNA expression in HNC tumor samples with different T-stage, N-stage, M-stage and grades. p< 0.05, ** p< 0.01, *** p< 0.001

differentiated samples (p< 0.02) compared to well and moderately differentiated tumors, as shown in Figure 3.

Correlation of UPR^{m} pathway genes expression with histopathological parameters

Spearmen correlation analysis was performed to associate the expression deregulation of UPR^{mt} pathway genes with different histo-pathological parameters of HNC patients. In gene to histopathological parameters, a significant negative association was observed between SIRT3 and T-stage (r= -0.343; p< 0.02), SIRT3 with N-stage (r= -0.295; p< 0.04) and SIRT3 with M-stage (r= -0.247*; p = 0.02). A significant positive association was observed between the FOXO3a and M-stage (r= 0.212*; p< 0.05), FOXO3a with grade (r= 0.437; p = 0.0008), SOD2 with N-stage (r= 0.529; p < 0.0004), SOD2 with M stage (r = 0.367; p= 0.004), SOD2 with grade (r= 0.520; p < 0.0004) and SOD2 with survival (r= 0.222; p< 0.04) in HNC patients as shown in Table 2.

In case of gene to gene correlation, negative association (significant) was observed between FOXO3a and SIRT3 (r= -0.699; p< 0.0002), SOD2 with SIRT3 (r= -0.372; p< 0.04) and Ki-67 with SIRT3 (r= -0.391; p< 0.03) in HNC patients. Furthermore, a significant positive association was observed between FOXO3a and SOD2 (r= 0.447; p< 0.0006), FOXO3a with Ki-67 (r= 0.364; p < 0.03) and SOD2 with Ki-67 (r= 0.288; p< 0.04) in HNC patients as shown in Table 2.

Kaplan-Meier analysis of Expression deregulation of UPRmt pathway genes

Kaplan-Meier was used to associate the expression level of un-folded protein response (UPRmt) genes with survival of head & neck cancer patients. Ka-

Table 2. Spearman correlations among clinical features and mitochondrial un-folded protein response (UPR^{mt}) gene expression of HNC †

T-stage	N-stage	M-stage	Grade	Type	survival	SIRT3	FOXO3a	SOD2	Ki-67
T-stage	**0.99*****	0.042	**0.231***	0.129	-0.004	**-0.343***	0.127	0.137	0.149
N-stage		0.060	0.200*	0.119	-0.001	-0.295*	0.009	0.529**	0.112
M-stage			0.163	-0.045	0.021	-0.247*	0.512**	-0.367**	0.071
Grade				0.028	0.111	0.249*	0.437**	0.520**	0.023
Tumor area					-0.189	0.078	0.134	-0.022	-0.169
Survival						-0.117	-0.252*	0.222*	0.103
SIRT3							-0.669**	0.372*	0.391*
FOXO3a								0.447**	0.364*
SOD2									0.288*

† Spearman correlation coefficients. The expression levels of SIRT3, FOXO3a, SOD2 and Ki-67 for the patient cohort were based on the relative mRNA level. The p values were computed using one-way ANOVA and χ^2-test. * p< 0.05, ** p< 0.01, *** p< 0.001

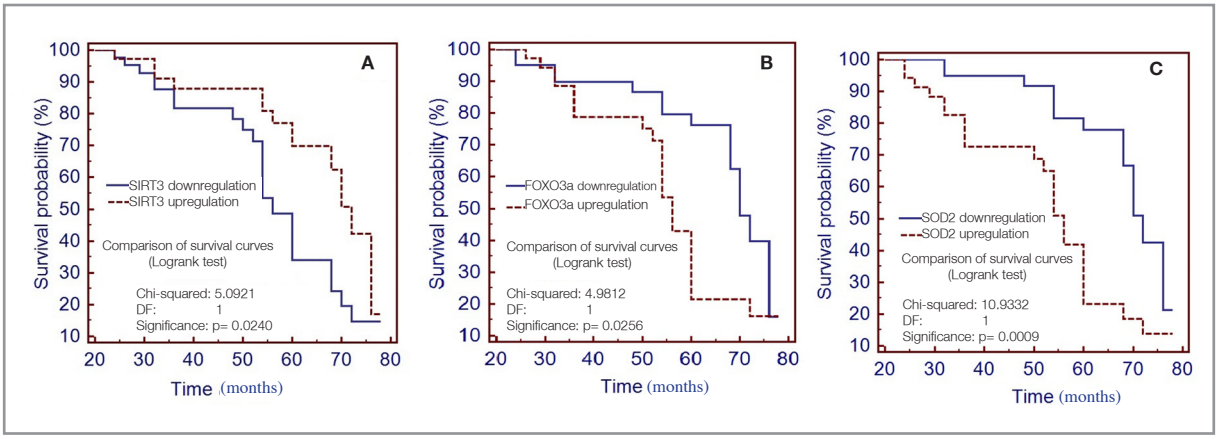

Figure 4. Kaplan-Meier analysis of (A) SIRT3, (B) FOXO3a, (C) SOD2 in HNC patients

plan-Meier analysis showed that down-regulation of SIRT3 gene was associated with decreased survival of HNC patients (logrank p= 0.02, HR= 1.90, 95% CI= 1.63-2.21) compared to up-regulation of SIRT3 gene as shown in Figure 4A. Up-regulation of FOXO3a (logrank p = 0.03, HR= 1.88, 95% CI= 1.41-2.49) and SOD2 (logrank p= 0.0009, HR= 2.54, 95% CI= 1.31-4.90) was also observed associated with decreased survival of HNC patients compared to down-regulation of FOXO3a and SOD2 gene, respectively, as shown in Figure 4B and 4C.

Cox Regression Analysis

Uni-variate Cox regression analysis was used for overall survival and is summarized in Table 3. Down-regulation of SIRT3 (HR= 1.90, 95%CI (1.63-2.21); p< 0.04) and up-regulation of FOXO3a (HR= 1.88, 95%CI (1.41-2.49); p< 0.03) and SOD2 (HR= 4.53, 95%CI (2.42-8.47); p< 0.001) was observed associated with decreased overall survival of HNC patients. Advance T-stage, N-stage and M-stage were found associated with worse overall survival in HNC patients as shown in Table 3.

Table 3. UPRm pathway genes and overall survival in HNC

Univariate analysis				Multivariate analysis			
Parameter	**HR**	**95% CI**	**p-value**	**Parameter**	**HR**	**95% CI**	**p-value**
SIRT3	1.90	1.63-2.21	0.04	SIRT3	5.25	2.13-12.88	0.0009
FOXO3a	1.88	1.41-2.49	0.03	FOXO3a	4.48	2.44-8.20	0.001
SOD2	4.53	2.42-8.47	0.001	SOD2	3.74	2.57-5.43	0.002
Age	0.775	0.71-0.84	0.457	T-stage	2.46	2.24-2.69	0.01
Gender	0.782	0.30-1.99	0.628	N-stage	2.40	2.08-2.75	0.01
Tumor area	0.88	0.58-1.10	0.346	M-stage	5.08	2.16-11.89	0.001
Smoking	0.952	0.78-1.15	0.294				
T-stage	1.785	1.35-2.34	0.04				
N-stage	1.713	1.20-2.44	0.04				
M-stage	2.150	2.11-2.18	0.03				
Grade	0.691	0.10-4.57	0.07				

HR= Hazzard ratio; CI= confidence interval; p-value ≤ 0.05 considered as statistically significant

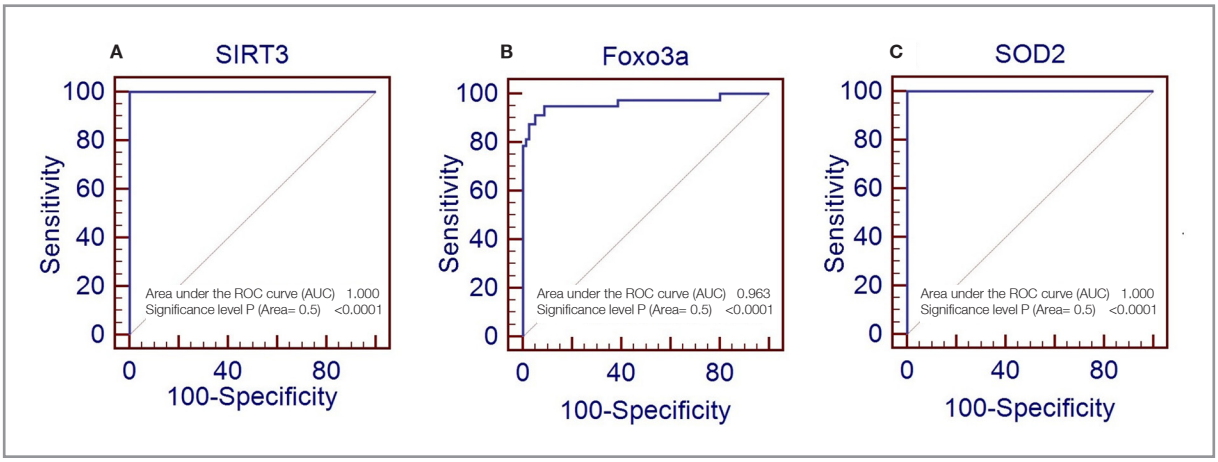

Figure 5. ROC curve anlysis of SIRT3 gene (A), FOXO3a (B) and SOD2 (C) in HNC patients

Multi-variate Cox Regression Analysis

Multi-variate Cox regression analysis was also performed and summarized in Table 3. Similar to univariate analysis, current analysis also showed that loss of SIRT3 (HR= 5.25, 95%CI (2.13-12.88); p< 0.0009) were found associated with worse overall survival of HNC patients. Additionally, increase in FOXO3a (HR= 4.48, 95% CI (2.44-8.20); p< 0.001) and SOD2 levels (HR= 3.74, 95% CI (2.57-5.43); p< 0.002) were also associated with worse overall survival of HNC patients and acts as independent poor prognostic factor in HNC as shown in Table 3. Furthermore, T-stage (HR= 2.46, 95% CI (2.24-2.69); p< 0.01), N-stage (HR= 2.40, 95% CI (2.08-2.75); p< 0.01) and M-stage (HR= 5.08, 95% CI (2.16-11.89); p< 0.001) were also independent prognostic factors as shown in Table 3.

ROC Curve Analysis

To assess the diagnostic value of UPR^{m} pathway genes, ROC curve analysis was performed as shown in Figure 5. After the generation of ROC curve, area under the curve (AUC) and 95% confidence interval (CI) was calculated. The area under the curve for SIRT3 gene was 1.00 (95% CI: 0.991-1.00; p< 0.0001), 96.0 (95% CI: 0.92 to 0.987; p< 0.0001) for FOXO3a gene, 1.00 (95% CI: 0.97 to 1.00; p< 0.0001) ROC curve analysis results for SOD2 gene are shown in Figure 5.

DISCUSSION

Genetic/epigenetic defects such as genomic alterations and expressional deregulations that are responsible for an increased risk of head and neck cancer (HNC) development have been evaluated in many earlier studies. However, contribution of mitochondrial genes' alternations in carcinogenesis has generally not been investigated.[4] Among these mitochondrial genes, the mitochondrial unfolded protein response (UPRmt) mechanism is stimulated in response to increased mitochondrial damage, increased reactive oxygen species (ROS) and elevated mitochondrial proteotoxic stress.[23,24] The UPRmt mechanism is controlled by mitochondrial sirtuin (SIRT3) which is responsible for the activation of anti-oxidant genes in mitochondria and elimination of irreversibly impaired mitochondria via mitophagy.[25] The SIRT3 axis of the UPRmt stimulates FOXO3a, which consequently results in the stimulation of manganese super-oxide dis-mutase (SOD2).[26] Sirtuin/FOXO/SOD2 axis of UPRmt mechanism has been deregulated in many cancers including breast cancer,[27] and gastric cancer.[28]

No or limited studies have been published elucidating the role of UPRmt mechanism and head & neck carcinogenesis. The aim of this study was to assess the expression deregulation of Sirtuin3/FOXO3a/SOD2 axis of UPRmt mechanism in HNC patients. The expression deregulation of selected genes (SIRT3, FOXO3a and SOD2) was also correlated

with histopathological parameters and survival status of HNC patients to figure out the prognostic value of Sirtuin/FOXO3a/SOD2 axis in HNC.

Significant down regulation of SIRT3 gene was observed in HNC patients compared to controls and this down-regulation was observed more pronounced in advanced TNM stage and advance grade of HNC compared to early TNM and early grade of HNC patients. Reduced levels of SIRT3 has already been reported in esophageal cancer,[29] breast cancer[30] and head & neck cancer.[31] SIRT3 appears to have dual role in the process of carcinogenesis, as a tumor suppressor and oncogene, depending on the cancer types.[32] The exact mechanism required to perform this dual function is still unclear. Several studies have reported that any damage in the function of proteins that are involved in fidelity such as SIRT3 create a cellular atmosphere that is tolerant for the development of disease, which can lead to human illnesses connected to aging such as carcinogenesis.[33,34] In many earlier studies the down-regulation SIRT3 has resulted in aberrant glucose metabolism[8,35] increased ROS,[36] mitochondrial membrane damage,[37] causing enhanced cellular proliferation, migration, and tumorigenesis.[38] However, some studies have reported that upregulation of SIRT3 in involved in reducing tumorigenesis by inhibition of glycolysis, proliferation and ROS creation by maintaining HIF-1a balance through its downstream signaling.[39,40] SIRT3 has also been reported to have a stress induced deacetylation activity whose upregulation is involved in protection of tumor cells from cell death induced by oxidative and genotoxic stress.[41] However, Chen et al (2014) reported that upregulation of SIRT3 in tumor cells may have a role in reduction of apoptotic activity and enhancement of survival signals to enhance tumorogenesis. Further studies may decipher the mechanism required for altered function of fidelity proteins.[42] Based on our results, we hypothesize that SIRT3 is a gnomically expressed, mitochondrial localized tumor suppressor protein.[43]

A key question in SIRT3 biology was then to determine the downstream targets that are aberrantly regulated when SIRT3 is deleted, resulting in the tumor-permissive cellular phenotype. We propose that FOXO3a and MnSOD may be among these proteins. In case of FOXO3a, significant up-regulation was observed in cancer samples compared to controls. Up-regulation was observed higher in advanced N stage, M stage and grade compared to early N stage, M stage and grade of HNC patients. Previous studies have reported the tumor suppressor role of FOXO3a in different cancers such as breast cancer,[44-45] prostate cancer,[20] ovarian cancer,[46] renal cancer[47] and colon cancer.[48] Tumor promotor role of FOXO3a has been reported in gastric cancer,[49] which showed involvement of FOXO3a up-regulation during invasion and metastasis process of tumorigenesis. Another study by Yu et al (2018) has also reported the upregulated expression of FOXO3a in colorectal cancer in response to stress conditions, hypoxia and oxidative stress and results upregulation of reactive oxygen species (ROS) scavengers, like mitochondrial peroxiredoxin III, Mn superoxide dismutase (SOD2) and catalase.[50] This antioxidant actions of FoxO3 can enhance the survival of drug-resistant tumor cells.[51] Although no or limited studies have been carried out with HNC patients, however, FOXO3 expression has been observed at protein level in nasopharyngeal tumor and down-regulation of FOXO3a protein has been reported compared to controls.[52] Variation in results in our study compared to previous studies, may be due to the;

(i) assessment of FOXO3a activity before and after patient treatment.

(ii) Environment dependent roles of FOXO3a in the heterogeneous tissues of HNC, which reveal different biological properties at different stages. The in-activation of FOXO3a in the initial stage of tumor development by enhanced signaling from growth factors may offer a proliferative advantage to cancer. However, in advanced stages, stress environments, such as hypoxia, serum deprivation and oxidative stress, may re-activate FOXO3a and thus improve survival of tumor cell.[53]

The third important gene in UPRmt pathway is SOD2 and significant up-regulation of SOD2 was observed in HNC compared to controls in our study. This up-regulation was higher in advance TNM stage and grade compared to early TNM stage and grade. Significant up-regulation of SOD2 has been reported in prostate,[54] colorectal,[55] brain,[56] oral[18] and gastric cancer.[57] An earlier study

conducted by Ye H et al.[58] has also demonstrated that up-regulation of SOD2 is associated with head & neck cancer. SOD2 has been reported as antioxidant enzyme with dichotomous role in process of carcinogenesis, behaving as tumor suppressor and tumor promotor. Down-regulation of SOD2 has been reported earlier during tumor initiation and in non-metastatic cancer cell lines.[59] However, up-regulation of SOD2 has been observed during metastasis and metastatic cancer cell lines.[60,61] Expression of SOD2 is lower during the early stages of tumor development, that may contribute towards tumor progression, activity/expression of SOD2 is higher in advanced stages of tumor which contributes in production of mitochondrial peroxidases via angiogenic and oncogenic pathway activation, that will ultimately help in invasion.[62] Transgenic mice were used to track the activity of SOD2 by luciferase activity in skin cancer model, it has been observed that expression of SOD2 was shifted to high level in advance stages from low expression of SOD2 in early stages of carcinoma.[63]

Furthermore, it has also been reported in earlier studies that increased activity of SOD2 changes the redox equilibrium towards a higher cellular steady-state H2O2 status in tumor cells.[59-61] This enhanced H2O2 can provoke oxidation and inactivate phosphatases, resulting in improved redox signaling[64] and invasion, migration and angiogenic pathway during tumor development.[65-67] In our study, deregulation of SIRT3/FOXO3a might result in up-regulation/hyperacytylation of SOD2, which ultimately results in increased redox level, ROS[36] and thereby facilitating the tumor development and progression of HNC.

In order to test the involvement of deregulated axis of SIRT3/FOXO3a/SOD2 in progression of HNC, survival analysis and Cox regression analysis was performed. Down-regulated expression of SIRT3 and up-regulated expression of FOXO3a and SOD2 was observed associated with poor survival of HNC and may act as independent prognostic factor. Kenny and Germain[4] has also reported that patients had poor disease free survival with upregulated levels of SIRT3 axis of the UPR^{mt} pathway. Additional analysis showed that T-stage, N-stage and M-stage of HNC patients also act as independent prognostic factors.

Conclusion

In conclusion, our results suggest that SIRT3 acts as a tumor suppressor and it also contributes via UPRmt mechanism by up-regulating the FOXO3a and SOD2 in head & neck carcinogenesis. The results of present study strongly support the hypothesis that invasion and metastatic potential of tumor cells increase due to activation of the SIRT3 axis of the UPRmt pathway. In particular, deregulated expression of SIRT3/FOXO3a/SOD2 axis is associated with poor survival of HNC patients and might possibly be used for HNC patient stratification in combination with other prognostic markers. Thus, the data presented in current study may offer a new orientation in HNC research and a prospect for the development of potential therapy.

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