Clinicopathological implications of glutathione peroxidase 3 downregulation through DNA hypermethylation in oral squamous cell carcinoma

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Clinicopathological implications of glutathione peroxidase 3 downregulation through DNA hypermethylation in oral squamous cell carcinoma

Directed by Professor In Ho Cha

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2016년 12월 정 재 승

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Clinicopathological implications of glutathione peroxidase 3 downregulation through DNA hypermethylation in oral squamous cell carcinoma

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(Directed by Professor In Ho Cha, D.D.S., Ph.D.)

Reactive oxygen species (ROS) have been shown to be involved in tumor initiation and progression via structural changes in cancer-related genes. Glutathione peroxidase 3 (GPX3), a member of the glutathione peroxidase family, is a major scavenger of ROS produced by normal metabolism or after oxidative damage to host cells. A possible tumor suppressor function of GPX3 and its downregulation caused by promotor hypermethylation have been investigated in various cancers.

In this study, we investigated the association between methylation status and GPX3 expression in oral squamous cell carcinoma (OSCC) and further investigated the clinicopathological significance of GPX3 expression in patients with OSCC who underwent long-term follow-up.

Promoter hypermethylation of GPX3 was frequently detected in both OSCC cell lines (100%) and tissue samples (75%). In contrast, only 33.3% of normal oral mucosa samples showed hypermethylation of GPX3. In OSCC cell lines, GPX3 expression was restored after 5-aza-2'-deoxycytidine treatment at both the mRNA and protein levels. GPX3 protein expression was significantly decreased in OSCC tissues (56.1%) compared to normal oral mucosa (100%). In patients with OSCC, GPX3 downregulation was significantly associated with histological grade (P=0.005), lymph node metastasis (P=0.001), vascular invasion (P=0.016), and poor prognosis (P=0.007). We concluded that promoter hypermethylation-induced GPX3 downregulation is significantly related to poor prognostic indicators and decreased overall survival in OSCC, and these findings provide further evidence for GPX3 as a novel diagnostic and prognostic biomarker in patients with OSCC.

Key words: GPX3 downregulation, promoter hypermethylation, molecular biomarker, OSCC

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I. INTRODUTION

Reactive oxygen species (ROS) are highly reactive, oxygen-containing chemical species, including free radicals(Cheeseman and Slater, 1993). ROS can be generated by endogenous and exogenous substances, and have a dual nature. ROS are necessary for the

normal physiologic functions of cells; however, excessive generation of ROS can induce various types of cellular damage. Moreover, excessive production of ROS has been shown to be involved in tumor initiation and progression via structural changes in cancer-related genes(Katakwar, et al., 2016).

In normal physiologic conditions, cells avoid ROS-induced damage by balancing the elimination and generation of ROS using a scavenging system. Non-enzymatic chemicals and enzymatic systems are involved in the ROS scavenging system. The glutathione peroxidase (GPX) family is the major enzymatic system of ROS scavenging, and these enzymes are widely distributed in various organs(Takebe, et al., 2002). GPX3, a secreted GPX enzyme, is detectable in plasma, thyroid colloid, and mucosal surfaces, and is also selectively expressed in various normal human tissues such as the gastrointestinal tract, epithelial cells of the oviducts, and the cervix(He, et al., 2011; Kohrle, 2005; Lapointe, et al., 2005; Schomburg and Kohrle, 2008; Zhang, et al., 2014). As one of the key antioxidative enzymes, GPX3 catalyzes the reduction of lipid peroxides and hydrogen peroxide. Hydrogen peroxide can regulate multiple biological characteristics of cancer cells such as proliferation, migration, invasion, angiogenesis, and apoptosis, and GPX3 is expected to have implications for malignant transformation and cancer progression via control of the hydroperoxide levels of the cells(El Haddad, et al., 2012). Some investigators have shown that the production of ROS increased after GPX3 silencing in both colon cancer cell lines and muscle stem cells(Barrett, et al., 2013; Mork, et al., 1998).

Recently, the role of GPX3 expression in cancer initiation and progression has been documented in various human cancers(Brigelius-Flohe and Kipp, 2009; He, et al., 2011; Yu, et al., 2007). Downregulation of GPX3 in melanoma cell lines can increase the invasive ability and motility of the cells(Saga, et al., 2008). Moreover, in prostate cancer, GPX3 overexpression can attenuate tumorigenic and metastatic activity both *in vitro* and *in vivo(Brigelius-Flohe and Kipp, 2009)*. Loss of GPX3 expression is significantly associated with poor prognosis in various cancers including gallbladder cancer, cervical cancer, multiple myeloma, gastric cancer, and melanoma(Chen, et al., 2016; Saga, et al., 2008; Yang, et al., 2013; Zhang, et al., 2010; Zhang, et al., 2014). GPX3 may function as a tumor suppressor in cancer progression. The tumor suppressor activity of GPX3 seems to relate to its ability to suppress the expression of c-met.

The mechanisms of regulation of GPX3 expression remain largely unknown. Oxidative stress can lead to transcriptional upregulation of GPX3 in patients with asthma, inflammatory bowel disease, and experimental colitis, as well as in the diabetic mouse heart(Comhair, et al., 2001; Hoffenberg, et al., 1997; Kaiser, et al., 2013; Ma, et al., 2003). However, GPX3 expression is usually downregulated by promoter hypermethylation in various cancers such as melanoma and cervical, gastric, and esophageal cancers(He, et al., 2011; Saga, et al., 2008; Tham, et al., 2002; Zhang, et al., 2014). Promoter hypermethylation resulting in consequent downregulation or silencing of tumor suppressor genes is a hallmark of human cancers and an essential part of malignant transformation and cancer progression(Iwata, et al., 2006; Peng, et al., 2012). Inactivation

of GPX3 via monoallelic hypermethylation has been found in benign precursor lesions of the esophagus, and inactivation by hypermethylation of both alleles has been detected in invasive carcinoma(Herman and Baylin, 2003). Promoter methylation may be a crucial cause of GPX3 downregulation in both precancerous lesions and cancers.

Oral cancer is the ninth most common cause of cancer-related death worldwide, with 529,000 new cases diagnosed globally in 2012, and 29,200 deaths(Thompson, 2014). Oral squamous cell carcinoma(OSCC) is the most common histological type of oral cancer. In the present study, we determined GPX3 expression and promoter hypermethylation in primary OSCC cell lines and tissues, and further investigated the clinicopathological significance of GPX3 expression in patients with OSCC.

II. MATRIALS AND METHODS

1. Patients and samples

This study included OSCC tissue samples obtained from 198 patients with OSCC who received surgical treatment at Yonsei University Hospital, Korea, between 1995 and 2010. The following clinical parameters were recorded: age, gender, lesion site, T stage, LN metastasis, histological grade, perineural invasion, and vascular invasion (Table 1). Follow-up for the cohort of patients was performed for at least 5 years. The study was approved by the Institutional Review Board of the Dental Hospital, Yonsei University Medical Center (IRB No.2-2011-0044).

Table 1. Clinicopathological characteristics of 198 patients with OSCC

Clinicopathological variables	No. of patients (%)
Total cases	198
Age, years	
Median age (range)	60(26-86)
≤60	100(50.5)
>60	98(49.5)
Gender	
Male	144(72.7)
Female	54(27.3)
Site	
Tongue	47(23.7)
Floor of mouth	9(4.5)
Retromolar trigone	26(13.1)
Gingiva	90(45.5)
Palate	2(1.0)
Lip	1(0.5)
Cheek	23(11.6)
T stage	
T1	24(12.1)
T2	56(28.3)
T3	14(7.1)
T4	104(52.5)
N stage	
N0	122(61.6)
N1	28(14.1)
N2	46(23.2)
N3	2(1.0)
Histologic grade*	
WD	50(25.3)
MD	115(58.1)
PD	33(16.7)
Perineural invasion	
Negative	173(87.4)
Positive	25(12.6)
Vascular invasion	
Negative	178(89.9)
Positive	20(10.1)

*WD: well differentiated; MD: moderately differentiated; PD: poorly differentiated

2. Immunohistochemistry

Formalin-fixed, paraffin-embedded archival OSCC tissue sections were used for immunohistochemistry. Mouse monoclonal antibody against GPX3 (1:200; Abcam, Cambridge, MA, USA) was used as the primary antibody for the staining. All sections were deparaffinized with xylene and rehydrated with graded alcohol. Following antigen retrieval with antigen retrieval buffer (Dako, Carpinteria, CA, USA), endogenous peroxidase activity was blocked with endogenous enzyme block solution (Dako). The sections were incubated with primary antibody at room temperature for 1h. Real Envision^{TM} HRP Rabbit/Mouse detection system (Dako) was used as the secondary antibody. Slides were visualized with 3,3'-diaminobenzidine (DAB) and then counterstained with hematoxylin. Staining of normal mouse kidney tissue samples was run in parallel as a positive control. For a negative control, the primary antibody was replaced by phosphate buffered saline. The scoring of protein expression was performed using the weighted histoscore method, as described previously.(Witton, et al., 2004) The intensity of tumor cell staining was scored as 0 (negative), 1 (light brown), 2 (brown), and 3 (dark brown). The final score was calculated as follows: total score = (0x percentage of negative cells) + (1x percentage of light brown staining cells) + (2x percentage of brown staining cells) + (3x percentage of dark brown staining cells). Patients were subdivided into two groups based on the total score: low (total score 0–100) and high (total score 101–300) expression groups.

3. Cell lines and cell culture

OSCC cell lines HSC-2, HSC-3 and CA9-22 were supplied by the Korean Cell Line Bank (Seoul, Korea), and YD-10B and YD-38 were previously established by the Oral Cancer Research Institute, Yonsei University, Korea.(E. J. Lee, et al., 2005) All OSCC cell lines were cultured in mixed medium that contained Dulbecco's Modified Eagle's medium (DMEM; Gibco BRL, Grand Island, NY, USA) and Ham's nutrient mixture F12 (Gibco BRL) medium (3:1 ratio) and maintained in a cell culture incubator (5% CO2) at 37°C. The supplement consisted of 10% fetal bovine serum (FBS; HyClone Laboratories, Inc., Logan, UT, USA), 100 U/mL of penicillin, 100 mg/mL streptomycin, $1x10^{-10}$M cholera toxin, 0.4 g/mL hydrocortisone, 5 μg/mL insulin, 5 μg/mL transferrin, and $2x10^{-11}$M triiodothyronine.

4. Immunocytochemistry in human OSCC cell lines

GPX3 protein expression was detected in OSCC cell lines by immunocytochemistry. Cell lines were cultured on glass coverslips containing 6-well plates. The cells were fixed with 95% ethanol before blocking with 5% bovine serum antigen at room temperature for 1h. After blocking of the endogenous peroxidase activity, the cells were incubated with primary antibody at room temperature for 1h. Real Envision™ HRP Rabbit/Mouse detection system (Dako) was used as the secondary antibody. Visualization was conducted with DAB and counterstaining was performed with hematoxylin.

5. Total RNA extraction and reverse transcription PCR analysis

Total RNA was purified from OSCC cell lines using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). After cDNA synthesis, conventional RT-PCR analysis was performed using AccuPower PCR PreMix (Bioneer Corp., Seoul, Korea) with an annealing temperature of 58°C. β-actin was used as the housekeeping gene. Oligonucleotide primers used for the PCR were: 5'-CAACCAATTTGGAAAACAGG-3' and 5'-GTGGGAGGACAGGAGTTCTT-3' for GPX3; and 5'-ATAGCACAGCCTGGATAGCAACGTAC-3' and 5'-CACCTTCTACAATGAGCTGCGTGTG-3' for β-actin. (Zhang, et al., 2014)

6. Treatment with 5-aza-2'-deoxycytidine (5-Aza)

OSCC cell lines (HSC-3, YD-10B, and YD-38) were treated with 5-Aza (Sigma-Aldrich, Inc., St. Louis, MO, USA), 10 μM, for 72h, with drug replacement every 24h. GPX3 mRNA and protein expression were detected in each group of cells by RT-PCR analysis and immunocytochemistry.

7. DNA extraction and methylation-specific PCR

Genomic DNA was extracted from 5 OSCC cell lines, 6 normal oral mucosa tissue samples, and 12 OSCC tissue samples using the QIAamp DNA mini kit (Qiagen GmbH, Hilden, Germany). Modification of DNA was performed using the EZ DNA Methylation kit (Zymo Research Corp., Irvine, CA, USA). The primers 5'-TATGTTATTGTCGTTTCGGGAC-3'

and 5'-GTCCGTCTAAAATATCCGACG-3' were used in methylation-specific amplification, and the primers 5'-TTTATGTTATTGTTTTGGGATG-3' and 5'-ATCCATCTAAAATATCCAACACTCC-3' were used in unmethylation-specific amplification. The annealing temperature of the PCR was 59°C.

8. Statistical analysis

The statistical analysis was performed using commercially available software (SPSS version 23.0; IBM Corp., Armonk, NY, USA) for statistical analysis. A value of $P<0.05$ was considered to indicate a statistically significant difference. The difference between GPX3 mRNA expression in normal oral mucosa and OSCC tissue samples was investigated with the Mann-Whitney U test. The chi-square test and Fisher's exact test were used to analyze the differences between groups of clinical samples. Survival analysis for patients with OSCC was performed with Kaplan-Meier analysis, and the differences were compared with the log-rank test.

III. RESULTS

GPX3 mRNA expression in OSCC cell lines

GPX3 mRNA expression was determined in 5 OSCC cell lines by RT-PCR analysis. In this study, GPX3 mRNA expression was observed in CA9-22 and HSC-2, but not in HSC-3, YD-10B, and YD-38 cell lines. Normal keratinocytes obtained from foreskin were used as a positive control (Figure 1).

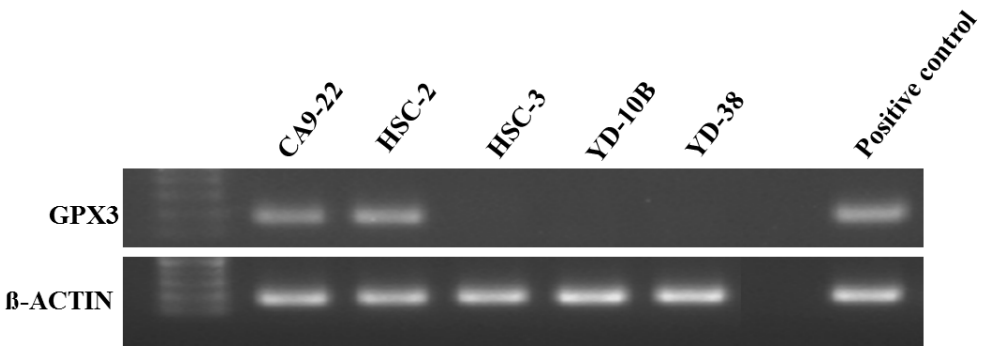

Figure 1. GPX3 mRNA expression was detected by RT-PCR analysis in 5 OSCC cell lines. Down-regulation of GPX3 expression was found in HSC-3, YD-10B and YD-38 cell lines.

GPX3 protein expression in OSCC cell lines

GPX3 protein expression was determined in 5 OSCC cell lines by immunocytochemistry. GPX3 protein expression was found in HSC-2 and CA9-22, but not in HSC-3, YD-10B and YD-38 cell lines, in the present study. Normal keratinocytes obtained from foreskin were used as a positive control (Figure 2).

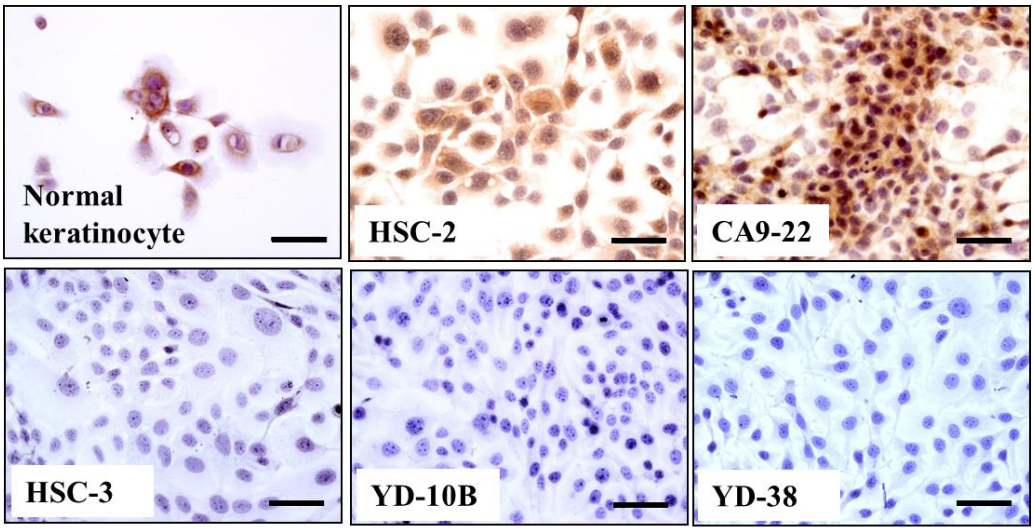

Figure 2. GPX3 protein expression in normal keratinocytes and OSCC cell lines. Down-regulation of GPX3 protein expression was found in HSC-3, YD-10B, and YD-38 cell lines. The original magnification of all figures was ×200. (Scale bar of all figures, 100 μm).

Promoter hypermethylation of GPX3 in OSCC cell lines

Promoter hypermethylation of GPX3 was determined in 5 OSCC cell lines. Methylation-specific amplification of GPX3 was found in all of the OSCC cell lines. Full methylation (presence of only methylated CpGs) was found in HSC-3, YD-10B, and YD-38, which showed negative GPX3 expression at both the mRNA and protein levels. Meanwhile, partial methylation (presence of both methylated and unmethylated CpGs) was found in both CA9-22 and HSC-2, which showed GPX3 expression at both the mRNA and protein levels. CpGnome Universal Methylated DNA (Chemicon International, Inc., Temecula, CA, USA) was used as the positive control (Figure 3).

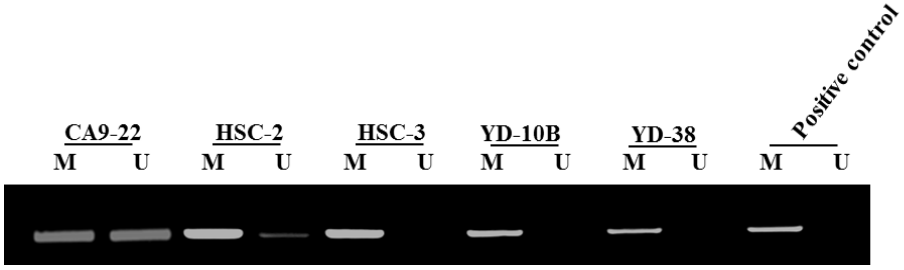

Figure 3. MSP analysis in OSCC cell lines. Methylation-specific amplification of GPX3 was found in all of the cell lines. Full methylation was found in HSC-3, YD-10B, and YD-38 cell lines, whereas CA9-22 and HSC-2 cell lines showed partial methylation of GPX3.

Restoration of GPX3 expression by 5-Aza treatment in OSCC cell lines

To investigate whether promoter hypermethylation of GPX3 can influence repression of GPX3 expression, 3 OSCC cell lines which showed full methylation for GPX3 were treated with 5-Aza, a demethylation agent. The results showed that both mRNA and protein expression of GPX3 were restored after 5-Aza treatment (Figure 4 A-B).

(A)

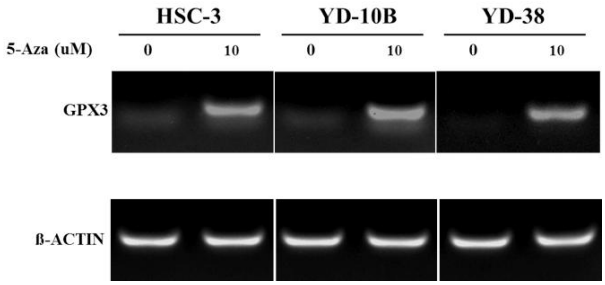

Figure 4A. GPX3 mRNA expression was detected in OSCC cell lines with or without 5-Aza treatment by RT-PCR analysis. GPX3 mRNA expression was restored after 5-Aza treatment in all 3 OSCC cell lines showed full methylation for GPX3.

(B)

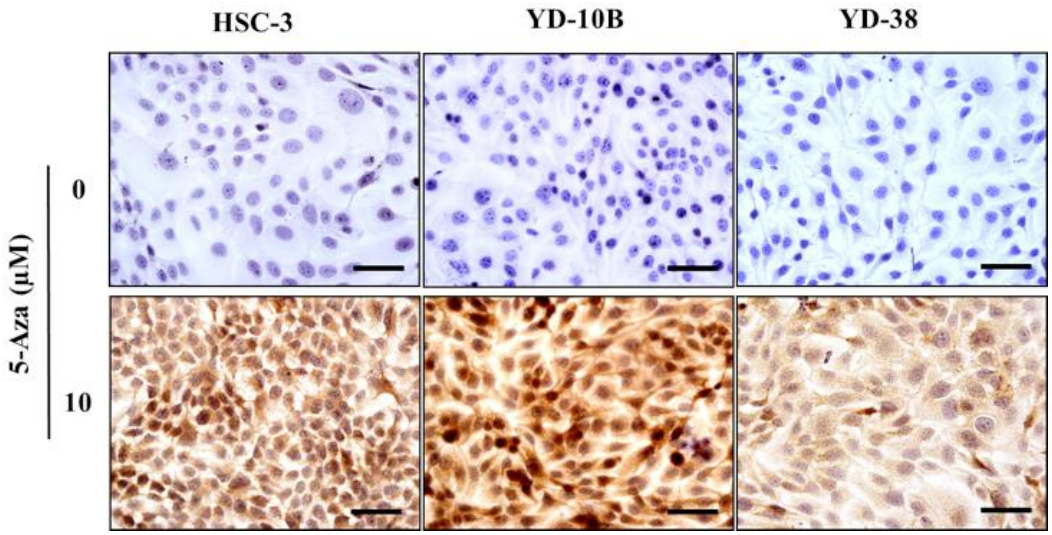

Figure 4B. GPX3 protein expression was detected in OSCC cell lines with or without 5-Aza treatment by immunocytochemistry. GPX3 protein expression was restored after 5-Aza treatment in all 3 OSCC cell lines showed full methylation for GPX3. (Original magnification: ×200; Scale: 100 μm).

Promoter hypermethylation of GPX3 in OSCC tissue samples

Promoter hypermethylation of GPX3 was also detected in normal oral mucosa and human OSCC tissue samples with or without GPX3 expression. Six samples were randomly selected from each group for MSP analysis. Unmethylation-specific amplification was more frequently detected in normal oral mucosa and OSCC tissues with GPX3 expression than in OSCC tissues without GPX3 expression. Moreover, methylation-specific amplification was detected more often in OSCC tissues without GPX3 expression than in normal oral mucosa and OSCC tissues with GPX3 expression. Four (66.7%) of the normal oral mucosa samples showed unmethylation for GPX3, and partial methylation was found in 2 (33.3%) cases. OSCC tissues without GPX3 expression showed full methylation in 4 (66.7%) cases and partial methylation in 2 (33.3%) cases. In contrast, OSCC tissues with GPX3 expression showed unmethylation in 3 (50%) cases and partial methylation in 3 (50%) cases (Figure 5).

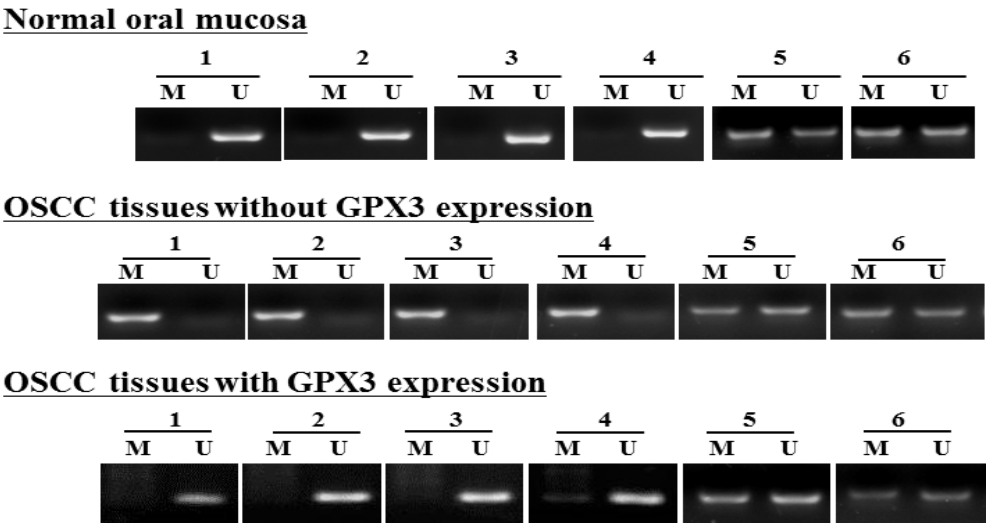

Figure 5. MSP analysis in 6 normal oral mucosa and OSCC tissue samples with (n=6) or without (n=6) GPX3 expression. Six samples were randomly selected from each group for MSP analysis.

GPX3 protein expression in normal oral mucosa and OSCC tissues

GPX3 expression was detected in the cytoplasm of normal epithelial cells of normal oral mucosa (Figure 6A-B) and cancer cells of OSCC tissues (Figure 6D-F). Cytoplasmic expression of GPX3 was detected in all normal oral mucosa samples and was significantly decreased in OSCC tissue samples (56.1%) (P<0.001) (Figure 7A). Moreover, high expression of GPX3 was also more frequently detected in normal oral mucosa tissues (83.3%) than in OSCC tissue samples (27.8%) in the present study (Figure 7B) (P<0.001).

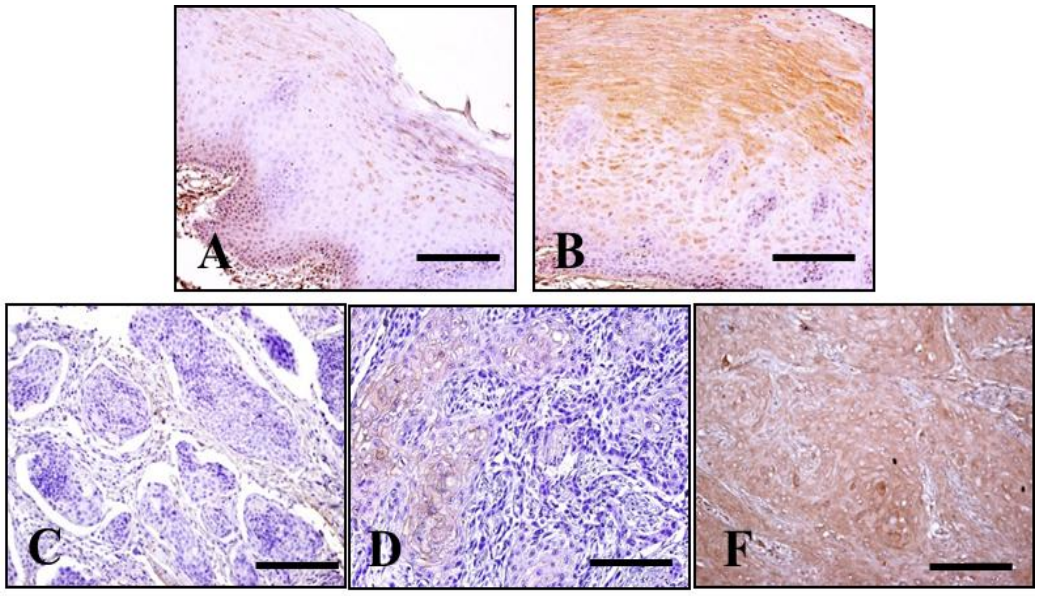

Figure 6. Protein expression of GPX3 in normal oral mucosa (A-B) and OSCC tissues (C, F). GPX3 expression was typically found in cytoplasm of normal epithelial cells and cancer cells. Low (A) and high (B) levels of GPX3 expression in normal oral mucosa. Negative (C), low (C, D), and high (F) GPX3 expression in OSCC tissues. (Original magnification, x200; scale bar, 100 μm).

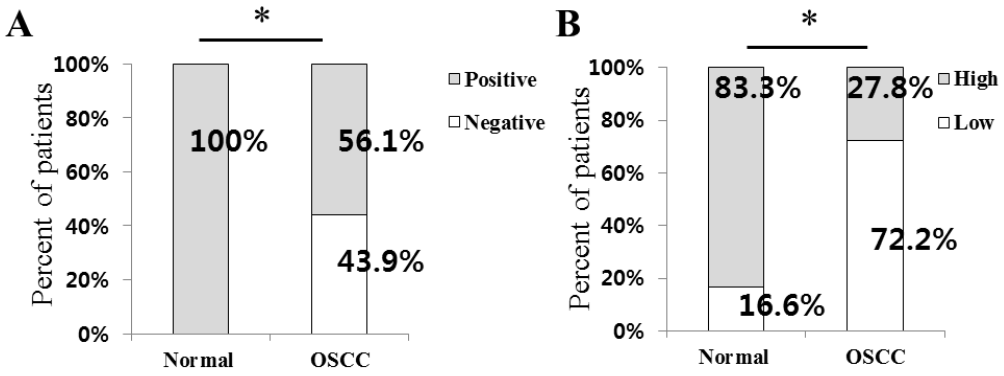

Figure 7. Frequency of GPX3 expression in normal oral mucosa and OSCC tissues. GPX3 expression was detected more often in normal oral mucosa than in OSCC tissues (A). High level of GPX3 expression was more frequently detected in normal oral mucosa than in OSCC tissue samples (B). The results were analyzed using Fisher's exact test (*P<0.001).

Clinicopathological significance of GPX3 expression in 198 patients with OSCC

GPX3 expression was more frequently detected in patients without LN metastasis (65.6%) than in patients with LN metastasis (40.8%) (P<0.001). Moreover, patients with poorly differentiated OSCC showed decreased GPX3 expression (30.3%) compared to patients with well differentiated (60.0%) or moderately differentiated OSCC (61.7%) (P=0.005). A high level of GPX3 expression was more frequently detected in patients without vascular invasion (30.3%) than in patients with vascular invasion (5.0%) (P=0.016) (Table 2).

Table 2. Clinicopathological significance of GPX3 expression in 198 patients with OSCC

Variables	Total	GPX3		*P*	GPX3		*P*
		Negative	Positive		Low	High	
T stage							
T1-T2	80	36(45.0)	44(55.0)	0.804	57(71.3)	23(28.7)	0.801
T3-T4	118	51(43.2)	67(56.8)		86(72.9)	32(27.1)	
N stage							
N0	122	42(34.4)	80(65.6)	0.001	79(64.8)	43(35.2)	0.003
N1-3	76	45(59.2)	31(40.8)		64(84.2)	12(15.8)	
Histologic grade							
WD	50	20(40.0)	30(60.0)		35(70.0)	15(30.0)	
MD	115	44(38.3)	71(61.7)	0.005#*	79(68.7)	36(31.3)	0.088
PD	33	23(69.7)	10(30.3)		29(87.9)	4(12.1)	
Perineural invasion							
Negative	173	73(42.2)	100(57.8)	0.194	122(70.5)	51(29.5)	0.16
Positive	25	14(56.0)	11(44.0)		21(84.0)	4(16.0)	
Vascular invasion							
Negative	178	75(42.1)	103(57.9)	0.127	124(69.7)	54(30.3)	0.016
Positive	20	12(60.0)	8(40.0)		19(95.0)	1(5.0)	

WD: well differentiated; MD: moderately differentiated; PD: poorly differentiated;#The results were analyzed by chi-squre test. *Signifiicant differences were found between WD versus PD and MD versus PD in GPX3 expression.

Survival analysis according to GPX3 expression in patients with OSCC

Survival analysis using the Kaplan-Meier method was performed to assess the prognostic significance of GPX3 expression in patients with OSCC. No significant difference in overall survival was found between patients with positive and negative GPX3 expression (Figure 8A). Patients with low expression of GPX3 showed poorer prognosis than patients with high expression of GPX3 (P=0.007, median survival duration 39.7 months for group with low GPX3 expression versus 51.1 months for group with high GPX3 expression) (Figure 8B).

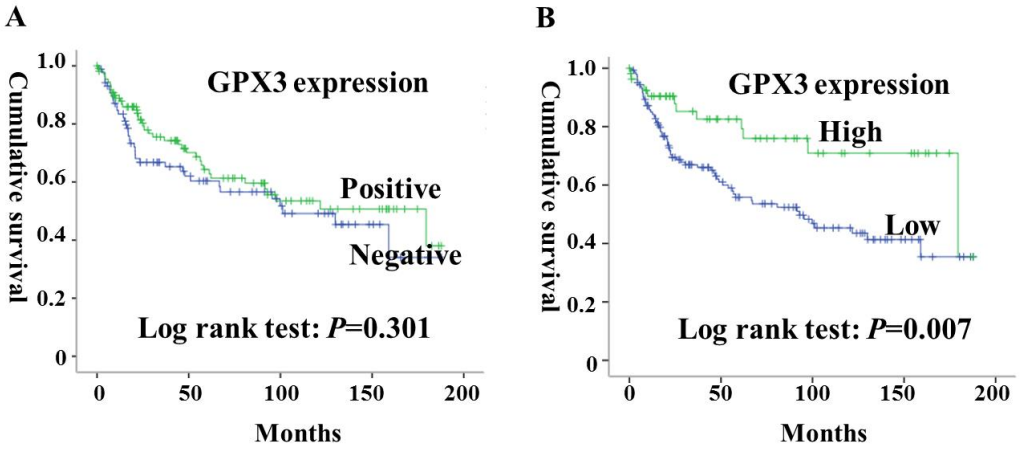

Figure 8. Overall survival analysis according to GPX3 expression in 198 patients with OSCC. The 198 patients with OSCC were classified into groups with negative (blue)/positive (green) (A) and low (blue) and high (green) GPX3 expression, and overall survival was analyzed by Kaplan-Meier analysis.

IV. DISCUSSION

DNA methylation, as one of the common physiologic epigenetic modulations, can regulate gene transcription(Baumgartel, et al., 2011; Deaton and Bird, 2011; Esteller, 2008). Aberrant methylation of DNA CpG regions, which silence tumor suppressor genes via hypermethylation and/or activate oncogenes via hypomethylation, is involved in various biological behaviors of cells, such as cell cycle signaling, DNA repair, and apoptotic pathways, and thereby contributes to malignant transformation and cancer progression(Esteller, 2008; Gerhauser, 2013; Jaenisch and Bird, 2003; Radhakrishnan, et al., 2011; Zeilinger, et al., 2013). In addition to its biological implications for malignant conversion, aberrant methylation is a useful biomarker for early identification and clinical assessment of various human cancers(Mulero-Navarro and Esteller, 2008). It is essential to identify novel and cancer-specific aberrant methylated genes for both mechanistic and translational investigations of human cancer(Baylin, 2005).

Both genetic and epigenetic aberrations are involved in molecular pathogenesis of the multistep progression. Recently, epigenetic repression of various tumor suppressor genes, such as p16, was reported in oral cancers, implying that epigenetic silencing of tumor suppressor genes is a crucial molecular mechanism in oral cancer carcinogenesis and progression(Lopez, et al., 2003; Radhakrishnan, et al., 2011; von Zeidler, et al., 2004).

Similar to other cancers, we found that GPX3 expression was downregulated by promoter hypermethylation at both the mRNA and protein levels in OSCC cell lines, but not in normal keratinocytes. GPX3 promoter hypermethylation was more frequently detected in OSCC tissues than in normal oral mucosa. Concordant with these findings, GPX3 protein expression was more frequently detected in normal oral mucosa tissue samples than in OSCC tissues. All of these results implied that GPX3 repression was also induced by epigenetic silencing in OSCC.

Interestingly, some normal oral mucosa samples also showed promoter methylation of GPX3 and demonstrated decreased GPX3 protein expression. Methylation patterns are related to environmental risk exposures such as tobacco, alcohol, and diet, in addition to an individual's genetic profile(Verma, 2012). Hypermethylation of p16 has also been detected both in normal oral mucosa and saliva DNA of smokers. GPX3 downregulation may also occur in the initial stages of carcinogenesis even in the absence of morphological changes. Some investigators have shown that methylation-induced GPX3 repression is a consistent and progressive molecular mechanism in Barrett's tumorigenesis. Compared to normal esophageal mucosa samples showing strong diffuse cytoplasmic expression for GPX3, both GPX3 promoter methylation and loss of GPX3 protein expression were more frequently found in Barrett's esophagus and dysplasia lesions, and became more so in esophageal adenocarcinoma lesions(O. J. Lee, et al., 2005). In the present study, the frequency of loss of GPX3 expression was increased in OSCC compared to normal oral mucosa. Additionally, GPX3 expression was significantly

decreased in patients with OSCC with LN metastasis or vascular invasion. The prominent influence of GPX3 expression on decreased cancer cell motility and invasion ability was previously reported in melanoma(Chen, et al., 2016). Moreover, a significant association between GPX3 expression and LN metastasis was also reported in various cancers including gastric cancer, cervical cancer, and melanoma(Chen, et al., 2016; Peng, et al., 2012; Zhang, et al., 2014). The prognostic significance of GPX3 methylation and protein expression has been investigated in various cancers, and GPX3 promoter methylation as well as loss of GPX3 expression are known as poor prognostic indicators(Chen, et al., 2011; Chen, et al., 2016; Zhang, et al., 2014). Similar results were also found in OSCC, in the present study. We found that patients with low levels of GPX3 expression showed poor prognosis than patients with high levels of GPX3 expression.

Our results showed that GPX3 downregulation was induced by promoter hypermethylation in OSCC, and this was significantly related to poor prognostic indicators and decreased overall survival of patients with OSCC. Our findings provide further evidence for GPX3 as a novel diagnostic and prognostic biomarker in OSCC. GPX3 may also serve as a possible therapeutic target for patients with OSCC.

V. CONCLUSION

The epigenetic inactivation of GPX3 is a frequent finding in OSCC progression and may be a crucial molecular mechanism in the pathogenesis of OSCC. Our findings provide further evidence for GPX3 as a diagnostic and prognostic biomarker in OSCC. GPX3 may also serve as a possible therapeutic target in patients with OSCC. However, at present, little is known about the role of GPX3 in OSCC. In the future, further studies will be needed to investigate the molecular mechanisms underlying the role of GPX3 inactivation in the pathogenesis and clinical assessment of OSCC.

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국문요약

구강편평세포암종에서 DNA hypermethylation에 의한 glutathione peroxidase 3 발현의 임상병리학적 의의

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정 재 승

활성산소는 암유전자의 구조적 변화를 일으켜 암의 발생과 진행에 관련이 되어있다. glutathione peroxidase 3(GPX3)는 glutathione family 중 하나이며 활성산소를 제거하는 주요 항 산화 효소이다. 다양한 암에서 promoter hypermethylation에 의한 GPX3 의 downregulation GPX3 의 tumor suppressor 로서의 가능성에 관한 많은 연구들이 있었다.

본 연구의 목적은 구강편평세포암종에서 methylation 정도와 GPX3 발현에 관한 상관관계를 보고, 구강편평세포암종 환자에서 GPX3 발현정도가 임상병리학적 예후에 미치는 영향을 보고자 하였다.

GPX3 promoter hypermethylation 은 구강편평세포암종 세포주와 조직에서 모두 높은 빈도(100%, 75%)로 관찰되었으나 정상 구강 점막조직에서는 낮은 빈도(33.3%)로 관찰되었다. 구강편평세포암종 세포주에서 demethylation agent 인 5-Aza 처리 후 GPX3 발현이 복원되었고, GPX3 downregulation 과 methylation 은 연관성을 가지고 있음을 확인하였다. 모든 정상 구강점막조직에서 GPX3 protein 발현이 관찰되었다. 반면에 구강편평세포암종 조직에서는 정상 구강점막조직에 비하여 GPX3 protein 발현이 통계학적으로 유의한 감소를 보였다. 구강편평세포암종 환자 군에서 GPX3 발현은 세포 분화도, 림프절 전이, 혈관침습 및 누적 생존률에서 각각 통계학적 유의성을 보였다.

결론적으로 promoter hypermethylation 에 의한 GPX3 downregulation 은 구강편평세포암종 환자의 진단 및 예후를 보는 생체표지자로의 의미가 있다고 할 수 있겠다.

핵심되는 말 : GPX 3 downregulation , promoter hypermethylation , 생체표지자, 구강편평세포암종