Particular gene upregulation and p53 heterogeneous expression in *TP53*-mutated maxillary carcinoma

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Abstract. It has been demonstrated that tumor protein p53 (*TP53*) mutation in maxillary squamous cell carcinoma, is more treatment-resistant compared with the carcinoma without *TP53* mutation. However, the association between *TP53* mutation and treatment resistance remains unclear. As a first step in understanding the biological differences between tumors with and without *TP53* mutation, a comprehensive gene expression analysis of maxillary squamous cell carcinoma with or without *TP53* mutation was performed. A total of 42 genes were identified to be differentially expressed by >4-fold. Quantification of their mRNA using quantitative polymerase chain reaction indicated 18 genes with high expression and three genes with low expression in *TP53* mutated tumors vs. *TP53* wild-type tumors. The 18 genes included eight cell adhesion (*DSC3, GRHL1, EPPK1, PROM2*, *ANXA8, DSP, JUP,* and *KRT6*B) and four cell growth inhibition (*SFN*, *CLCA2*, *SAMD9* and *TP63*) genes. Among these genes, *DSC3*, *SFN*, and *CSTA*, whose expression was markedly increased, also demonstrated high protein expression in immunohistochemical staining of *TP53* mutated tumors. The *TP53* mutated tumors demonstrated high nuclear staining of the TP53 protein only in tumor cells at the tumor margins adjacent to the stroma, whereas the tumor interior was negative for TP53. However, all tumor cells of *TP53* wild-type tumors exhibited positive nuclear staining for the TP53 protein. The combined findings suggest that *TP53* mutated tumors possess a phenotype opposite to that associated with cancer progression and malignant transformation, and exhibit tumor cell heterogeneity between the tumor interior and margins.

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Abbreviations: PCR, polymerase chain reaction; CDDP, cisplatin; TP53, tumor protein p53; HNSCC, head and neck squamous cell carcinoma; TP63, tumor protein p63; IHC, immunohistochemistry; CSTA, cystain A; SFN, stratifin; DSC3, desmocollin 3; DSP, desmoplakin; JUP, junction plakoglobin; ECM, extracellular matrix

Key words: gene expression, maxillary cancer, microarray, mutation, *TP53*

Introduction

Maxillary cancer is commonly treated with cisplatin (CDDP) chemotherapy combined with radiotherapy. Although the tumor regresses during treatment, in about half of the cases the tumor histopathologically remains, and ultimately the CDDP-resistant tumor is surgically resected. CDDP plays a central role in treatment of maxillary cancer; however, the existence of a CDDP-resistance mechanism has now been recognized (1). To examine whether known chemotherapy-resistant genes are involved in CDDP resistance of maxillary carcinoma, we previously analyzed gene expression in maxillary squamous cell carcinoma biopsies prior to treatment. The results showed that expression of a group of genes (multidrug resistance protein 1; multidrug resistance associated protein 1; Cu^{++} transporting, beta polypeptide; xeroderma pigmentosum; complementation group A; excision repair cross-complementing rodent repair deficiency, complementation group 1; B-cell CLL/lymphoma 2) associated with treatment resistance was decreased in these tumors, and that only tumor protein p53 (*TP53*) mutation was linked to treatment resistance (2). *TP53* mutations are associated with treatment resistance not only in head and neck cancers, but also in breast cancer, lung cancer, hepatic cancer, and chronic lymphocytic leukemia (3-8). On the other hand, it has been reported that there is no such association in small cell lung cancer or epithelial ovarian cancer (9,10). Thus, the relationship between *TP53* mutation and treatment resistance is not necessarily clear.

Recently, whole exome sequencing has shown major driver genes in head and neck squamous cell carcinoma (HNSCC). In addition to the previously identified *TP53*, cyclin dependent kinase inhibitor 2A, Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha, and histidyl-tRNA synthetase genes, mutations in major genes that regulate squamous differentiation, including notch1, interferon regulatory factor 6, and tumor protein p63 (*TP63*), have been newly identified as drivers (11,12). In particular, *TP53* mutations occur at a high frequency in HNSCC, but many non-*TP53* mutated tumors are human papillomavirus-positive (13). Both types of tumors may

involve a common mechanism mediated by *TP53* dysfunction, but the biological differences between these cancers are unclear. As a first step in understanding the biological differences observed between tumors with and without *TP53* mutation, this study aimed to clarify differences at the gene expression level between maxillary cancers with and without *TP53* mutation.

Materials and methods

Samples. Specimens were used from 14 patients with maxillary cancer (Table I). Tumor staging and differentiation was in accordance with the Union for International Cancer Control TNM classification (14). Maxillary cancer biopsy specimens before treatment were used in the study. This study was approved by the Ethics Committee at Nihon University School of Medicine and conforms to the Declaration of Helsinki (2013). Informed consent was obtained from all patients.

TP53 mutation analysis. Total RNA was extracted as described previously, and was used as a template for cDNA synthesis (2). The synthesized cDNA was used to perform polymerase chain reaction (PCR) analysis of a high mutation region (aa115-aa342) of the *TP53* gene as described previously. The sequence of the PCR products was analyzed by Sanger sequencing (2).

Comprehensive gene expression analysis. Comprehensive gene expression analysis was performed in 5 patients each with and without *TP53* mutations (Table I). Biotin-labeled cRNA was synthesized from total RNA according to the Affymetrix manual. Hybridization was performed using a GeneChip Human Genome U133 Plus 2.0 Array (Affymetrix, Santa Clara, CA). A GeneChip Fluidics Station 400 (Affymetrix, Inc., Santa Clara, CA, USA) and Scanner 3000 (Affymetrix, Inc.) were used for detection. Analysis was performed using GeneChip Operating Software (Affymetrix, Inc.) and GeneSpring v7 (Silicon Genetics, Redwood City, CA, USA); the output data were normalized per chip and per gene. Genes with >3-fold differential expression between TP53 mutation (+) and (-), that were commonly identified using two parametric tests (Student's t-test and Welch's t-test), were used as gene candidates with differential expression (Fig. 1).

Quantification of mRNA. A quantitative PCR (qPCR) assay was carried out using the SYBR-Green Real-time PCR Master Mix (Life Technologies, Frederick, MD, USA) as described previously (2). The gene expression level was normalized against glyceraldehyde-3-phosphate dehydrogenase *(GAPDH)* mRNA. Table II lists the primer sequences used.

Immunohistochemistry (IHC). Biopsy tissue was fixed in formalin and embedded in paraffin, and then 4-μm thin-sections were prepared. Four cases with p53 wild-type (1T, M11, 7T, M5) and 4 cases with a p53 mutation (M9, M8, M13, 8T) were used. After deparaffinization and removal of endogenous peroxidase, antigen activation was performed using citrate buffer (pH 6) in a 600 W microwave oven for 5 min [cystain A (CSTA), stratifin (SFN) or an autoclave for 5 min desmocollin 3 (DSC3)].

Table I. Clinicopathological features and *TP53* mutation of 14 cases of maxillary squamous cell carcinoma.

Case	Age	Sex	Stage[b]	Grade[b]	*TP53* mutation[c]
1T	46	F	II	2	
M6[a]	55	M	III	1	
M2[a]	55	M	III	2	
M11[a]	58	M	III	2	
7T[a]	60	M	III	2	
M5[a]	64	M	III	3	
M9[a]	73	M	III	1	P190T
M8	64	M	III	1	F285K
M1[a]	63	M	III	2	R280S
M10	64	M	III	2	c.782+1G>A
M13[a]	51	M	III	3	R156AfsX14
M12[a]	80	M	IV A	2	c.782+1G>A
M14[a]	67	M	IV A	2	Y220C
8T	65	M	IV A	2	H193R

[a]Cases used for comprehensive gene analysis. [b]Based on TMN Classification of Malignant Tumors (7th edition) (14). [c]Deletion of wild-type sequence only in case M9.

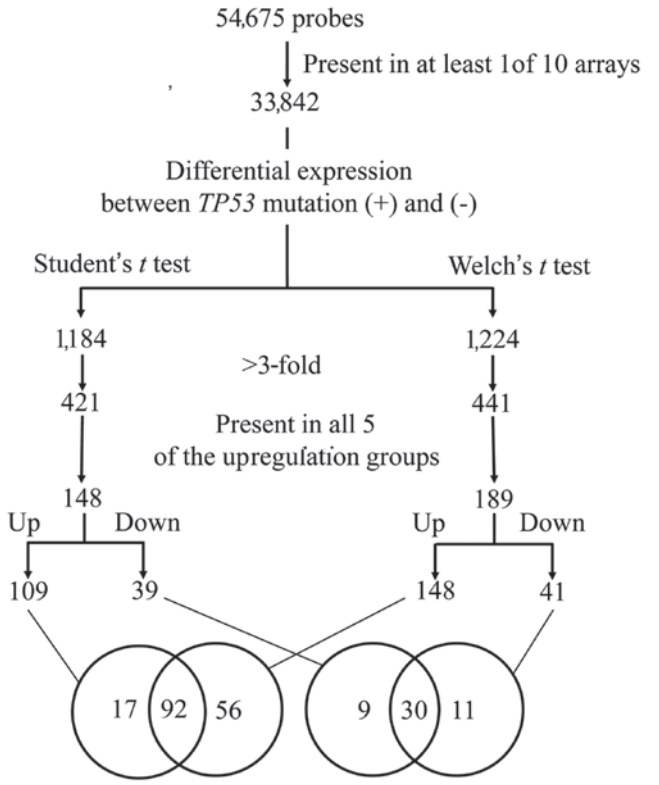

Figure 1. Flow sheet of comprehensive analysis of gene expression in maxillary squamous cell carcinoma with and without *TP53* mutation. Comparison was made between the two groups with and without *TP53* mutations using 5 microarrays of each group. There were 33,842 probes with a flag present on at least one of the 10 microarrays. The number of probes with ≥3-fold differential expression between the two groups was 421 probes by Student's t-test and 441 probes by Welch's t-test. The number of probes with a flag present on all 5 microarrays for >3-fold differential expression was 148 and 189, respectively. After checking for overlap, there were 92 probes indicating higher expression and 30 probes indicating lower expression of genes in *TP53* mutated tumors compared to non-*TP53* mutated tumors.

Table II. Primer sequences used for quantitative polymerase chain reaction analysis in this study.

Gene	Primer sequence	
	Forward (5'-3')	Reverse (5'-3')
CSTA	ACGGAAAATTGGAAGCTGTG	TTTGTCCGGGAAGACTTTTG
SFN	CAGGCTACTTCTCCCCTCCT	TCAATCTCGGTCTTGCACTG
DSC3	ATTGCAGTCTTGATTCTGCC	ACGTTTGTAGGGGAGCACAC
GRHL1	GCTAGTATCAGTCAGATGCA	GAAGGCTCTGATGCGTGATA
EPPK1	TCAGCTCAGCCATAATCACG	ACATGGCCTGGTAGATGCTC
PROM2	CTGATCCCCAGCATCATCTT	ACCAGATCACTCCCACAAGG
ANXA8	AGCTGGTCACAGAGTCTCCT	GCTGCTGAAGGATGTGTGTT
CLCA2	TACCTCTTGCTATTTTGTTA	GCTGCTTTGATGGGAGTAGA
SAMD9	GACATTATGGGCCTGGAAGT	TGTGAATTTCCCCTTTCTGG
PRRG4	AATATTTGTCAGTGCTTAAC	AAATGACCACACAGGCAGAA
DSP	TAGGAGAAAATTACCCTCCC	GAAAAGATTGCCGCTGTCAT
F2RL1	GCCACTTAGAATAGCATTTG	GATGTGGTCCAAACCCTCTG
S100A2	ATGAGTGGGAATGGCAAGAG	GCAGAGACAGACCCAGGAAG
MAST4	TCTCCTCTCTGTGGGAAGGA	GCCATCTTTGTGGTTCGTTT
JUP	AACCAGCTGTCGAAGAAGGA	GTGTCCAGGTCGCTGGTATT
SCD	TGTTCGTTGCCACTTTCTTG	TAGTTGTGGAAGCCCTCACC
TP63	GAGGTTGGGCTGTTCATCAT	GAGGAGAATTCGTGGAGCTG
KRT6B	TGCGAATGTCCTTTTTAGTT	TAATGGGCAGGATGGTTAGC
SFRP4	GACTTCCGACTTCCTTACAG	TCTGTACCAAAGGGCAAACC
HMCN1	ATCAGCTGAACCACTTATGA	AAACCAAACCTGTCCCACTG
MEST	GAATCGATCTGGTCGGCTTA	CATCAGTCGTGTGAGGATGG
GAPDH	GGTCGGAGTCAACGGATTTG	GGATCTCGCTCCTGGAAGAT

The primary antibodies used were rabbit polyclonal anti-human CSTA IgG (0.1 μg/ml, HPA001031; Atlas Antibodies AB, Stockholm, Sweden), mouse monoclonal anti-human 14-3-3 sigma IgG1 clone 1.N.6 (1 μg/ml, GTX14123; GeneTex, Irvine, CA, USA), mouse monoclonal anti-human desmocollin 3 IgG1 clone Dsc3-U114 (0.05 μg/ml, 61093; Progen Biotech, Heidelberg, Germany), and mouse monoclonal anti-human p53 IgG2b clone DO-7 (0.69 μg/ml, M7001; Dako, Glostrup, Denmark). The reactions were carried out overnight at 4˚C. A Histofine Simple Stain MAX-PO (R) kit or MAX-PO (M) kit (Nichirei, Tokyo, Japan) was used for secondary antibodies. The sections were colored with diaminobenzidine and nuclei were stained with hematoxylin.

Statistical analysis. Age, stage, grade, and mRNA expression levels were compared between the two groups with and without *TP53* mutation using the Mann-Whitney U test. P<0.05 was considered to indicate a statistically significant difference.

Results

Clinicopathological features of maxillary carcinoma with and without TP53 mutation. Eight of the 14 patients had a *TP53* mutation (Table I). These mutations included 5 point mutations, 2 splicing abnormalities, and one frameshift mutation. Table III compares the clinicopathological features of patients with and without *TP53* mutations. Tumor stage and grade were not significantly related to *TP53* mutation status. However, there was a correlation between *TP53* mutations and age; thus, *TP53* mutation-positive patients were significantly older than those without *TP53* mutation (P=0.0273). *TP53* mRNA expression levels did not significantly differ between the two groups (2).

Table III. Comparison of the clinicopathological features of maxillary squamous cell carcinoma with and without *TP53* mutation.

Feature	*TP53* mutation		P-value[b]
	(-)	(+)	
Age[a]	56.3±6.1	65.9±8.4	0.0273
Stage			0.0611
II	1	0	
III	5	5	
IVA	0	3	
Grade			0.7047
1	1	2	
2	4	4	
3	1	1	

[a]Mean ± standard deviation, [b]Two-group comparison using the Mann-Whitney U test.

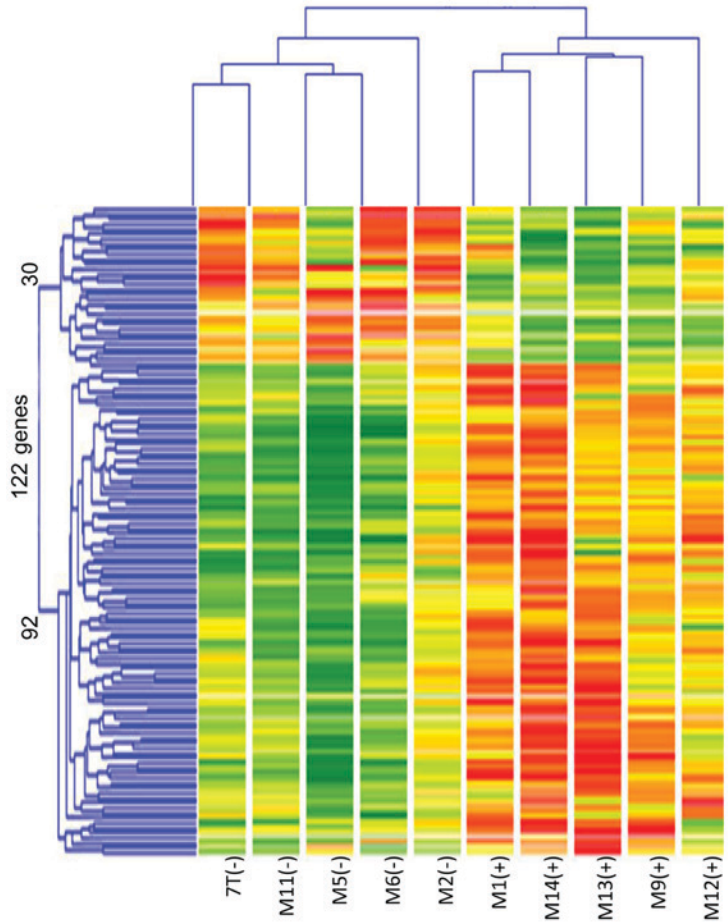

Figure 2. Cluster analysis of 10 maxillary squamous cell carcinomas by their expression profiles of 122 genes. Cluster analysis of 10 maxillary squamous cell carcinomas based on the gene expression pattern of 122 genes with ≥3-fold differential expression in *TP53* mutated tumors vs. non-mutated tumors (Gene Spring v7). Minus or plus indicates the absence or presence of *TP53* mutation. Red and green colors indicate high and low expression values, respectively.

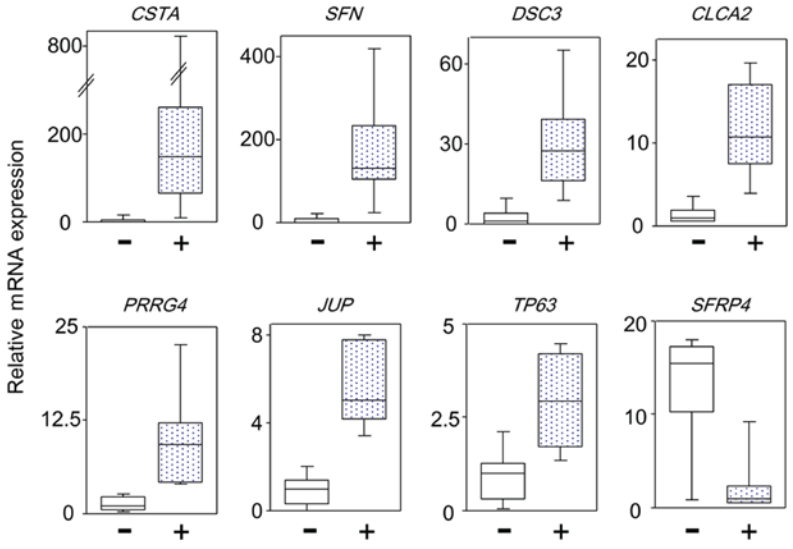

Figure 3. The mRNA expression of 8 representative genes in maxillary squamous cell carcinoma with (+) and without (-) *TP53* mutation is shown. Relative mRNA expression is shown, where the mRNA expression of the median level in the low expression group is assigned a value of 1. The mRNA levels of these 8 representative genes were significantly different between the 2 groups by the Mann-Whitney U test. Each fold-change of difference and the P-value are shown in Table IV.

Differential gene expression in maxillary carcinoma with and without TP53 mutation. Comprehensive gene expression analysis was performed with 10 microarrays using mRNA from the maxillary cancer specimens. The results showed 92 genes in *TP53* mutated tumors with ≥3-fold increased expression and 30 genes whose expression was decreased to approximately

Table IV. Twenty-one validated genes with differential expression in *TP53* mutated versus non-mutated tumors.

A, Genes upregulated in TP53 mutated cancer

Gene symbol	Gene title	FC[a]	P-value[b]	Function
CSTA	Cystatin A	148.8	0.0174	Cysteine protease inhibitor
SFN	Stratifin	129.9	0.0106	Cell cycle arrest Tumor progression
DSC3	Desmocollin 3	27.3	0.0106	Desmosome
GRHL1	Grainyhead-like 1	20.1	0.0249	Transcription factor Cell adhesion
EPPK1	Epiplakin 1	19.9	0.0096	Cell matrix adhesion
PROM2	Prominin 2	17.2	0.0176	Membrane glycoprotein
ANXA8	Annexin A8	11.7	0.0106	Adherens junction
CLCA2	Chloride channel accessory 2	10.7	0.0106	p53-inducible senescence
SAMD9	Sterile alpha motif domain containing 9	9.5	0.0176	Regulation of cell proliferation
PRRG4	Proline rich Gla 4	9.3	0.0062	Unknown
DSP	Desmoplakin	9.3	0.0285	Desmosome
F2RL1	Coagulation factor II receptor-like 1	7.5	0.0062	Pro-inflammation
S100A2	S100 calcium binding protein A2	7.2	0.0176	Tumor suppressor or promoter
MAST4	Microtubule associated serine/threonine kinase family member 4	7.1	0.0456	Unknown
JUP	Junction plakoglobin	5.1	0.0007	Desmosome
SCD	Stearoyl-CoA desaturase	3.2	0.0446	Fatty acid biosynthesis
TP63	Tumor protein p63	2.9	0.0200	ΔNp63: Cell growth Tap63: Apoptosis
KRT6B	Keratin 6B	2.7	0.0285	Intermediate filament cytoskeleton

B, Genes downregulated in TP53 mutated cancer

Gene symbol	Gene title	FC[a]	P-value[b]	Function
SFRP4	Secreted frizzled-related protein 4	-15.4	0.0112	Regulation of Wnt signal
HMCN1	Hemicentin 1	-8.5	0.0285	Extracellular matrix
MEST	Mesoderm specific transcript homolog	-6.7	0.0104	α/β-fold hydrolase (imprinting gene)

[a]Fold-change (FC). [b]P-value, comparison between expression in TP53+ and -mutated cancer groups using the Mann-Whitney U test.

1/3 compared to non-*TP53* mutated tumors (Fig. 1). Cluster classification was performed in 10 cases based on the expression pattern of these 122 genes. As shown in Fig. 2, the 10 cases could be accurately classified into two clusters based on *TP53* mutation status. To confirm these gene expression levels, qPCR analysis of the mRNA expression of 42 genes with ≥4-fold differential expression between *TP53* and non-*TP53* mutated tumors based on the microarray results was performed using patient maxillary squamous cell carcinoma samples. Twenty-one genes with significant differences in expression between the two groups were identified. There were no significant differences in the expression level of the remaining 21 genes due to the different normalization methods used. The differential genes commonly identified by different normalization methods are considered to be reliable. Fig. 3 shows representative results of the differential gene expression. Table IV lists 18 genes with high expression and 3 genes with low expression in *TP53* mutated tumors compared to non-*TP53* mutated tumors. The 18 genes included 8 cell adhesion genes (*DSC3;* grainyhead like transcription factor 1; epiplakin 1; prominin 2; annexin A8; desmoplakin (*DSP*); junction plakoglobin (*JUP*); and keratin 6B) and 4 cell growth inhibition genes (*SFN,* chloride channel accessory 2, sterile alpha motif domain containing 9, and *TP63*). Thus, in *TP53* mutated tumors, the expression of genes that inhibited proliferation, invasion, and metastases was unexpectedly increased, compared to wild-type tumors.

IHC analysis of DSC3, CSTA, SFN and TP53. IHC analysis of the 3 genes with the highest differential expression between *TP53* mutated and non-mutated tumors was performed. Fig. 4 shows representative staining images. CSTA was negative in normal paranasal sinus mucosa, and staining was also weak

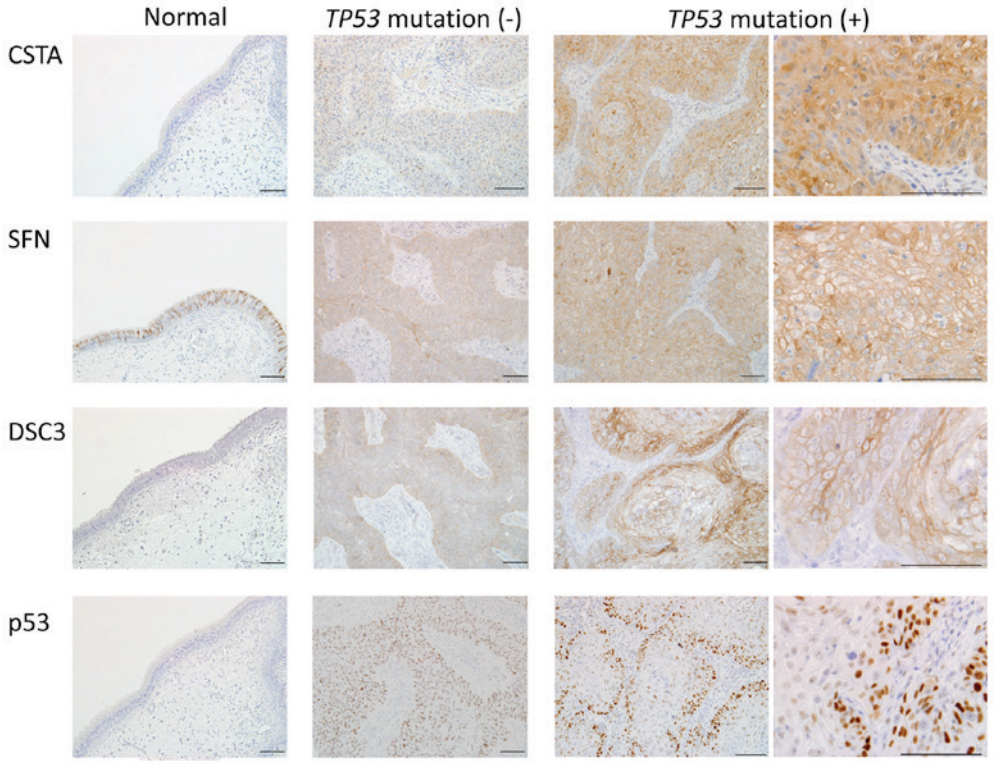

Figure 4. Representative IHC analysis of CSTA, SFN, DSC3, and p53 expression in maxillary squamous cell carcinoma with and without *Tp53* mutation. Normal, normal paranasal sinus mucosa; *TP53* mutation (-), case 1T; *TP53* mutation (+), case 8T (CSTA, DSC3) and M9 (SFN). Bar, 100 μm.

in the *TP53* wild-type tumors. In *TP53* mutated tumors, CSTA was strongly expressed throughout the entire tumor. SFN was strongly stained in cell membranes in normal paranasal sinus mucosa. Staining of SFN was stronger in mutated tumors than in the wild-type tumors, and was localized more in the cell membrane than in the cytoplasm. DSC3 expression was weakly positive in cell membranes in normal paranasal sinus mucosa. Staining in tumors was stronger in cell membranes, with stronger staining in mutated tumors compared to wild-type tumors. For all these genes, the genes were strongly expressed at the protein as well as at the mRNA level in *TP53* mutated tumors.

Localization of the p53 protein was also examined using IHC. p53 expression was negative in normal paranasal sinus mucosa, whereas in *TP53* wild-type tumors, positive p53 nuclear expression was observed in all cancer cells. Immunostaining of *TP53* mutated tumors did not show p53 staining throughout the tumor; instead, strong nuclear p53 staining was only observed in tumor cells in the margins adjacent to the stroma. The stroma and tumor interior were p53 negative. Even in biopsy specimens after treatment, p53 staining in residual tumor was observed in the tumor margins (data not shown). p53 IHC staining was negative in case M13 (R156AfsX14) because of the frameshift mutation.

Discussion

This study found clear differences in gene expression between *TP53* mutated and *TP53* wild-type maxillary squamous cell carcinoma tumors. A characteristic finding was increased expression of cell growth inhibition genes and increased expression of cell adhesion genes such as DSC3 in the *TP53* mutated tumors. Takahashi *et al* compared gene expression using microarrays in breast cancer with and without *TP53* mutations (15). They found that the expression of genes that stimulate the cell cycle and cell division was increased in *TP53* mutated tumors, thus suggesting that *TP53* mutation was a poor prognostic factor in breast cancer. However, our results were the opposite; i.e., we found increased expression of 8 tumor suppressor genes including SFN in *TP53* mutated tumors compared to wild type tumors. In particular, there was ≥100-fold differential expression of CSTA and SFN based on *TP53* mutation status (Table IV).

CSTA is a cysteine protease inhibitor that specifically inhibits cathepsin B (16). Cathepsin B, because it localizes on tumor cell surfaces and degrades the extracellular matrix (ECM), is involved in cancer progression (17). Our study showed markedly increased expression of CSTA in *TP53* mutated tumors, with strong expression in the cytoplasm. This result suggested that the overexpressed CSTA might more efficiently inhibit ECM degradation, resulting in a decrease in cancer progression of *TP53* mutated tumors. Increased CSTA has been shown to inhibit the migration, invasion, and proliferation of laryngeal cancer (18). CSTA expression has been reported to reduce distant metastases in breast cancer, and this may be due to inhibition of cysteine cathepsins (19). On the other hand, increased CSTA was found to be associated with a poorer prognosis in nasopharyngeal cancer (16). Thus, the effects of CSTA differ depending on the type of tumor, and in our study, CSTA may have similar effects as those reported for nasopharyngeal cancer.

SFN is a gene that is induced by TP53 and is also called the 14-3-3 σ protein. SFN obstructs G2 cell cycle entry by sequestering Cdc2-cyclin B and Cdc/Cdk complexes in the

cytoplasm (20,21). Based on these functions, it was surmised that SFN is a tumor suppressor protein. In addition, 14-3-3 σ opposes tumor-promoting metabolic programs by enhancing c-Myc poly-ubiquitination and subsequent degradation. Thus, cancer metabolic reprogramming occurs in tumors with low 14-3-3 σ expression (22). However, tumors with high SFN expression have also been reported (23-26). Cancers with high SFN expression have increased proliferation and anti-cancer drug resistance and thus SFN can be regarded as a tumor progressive protein (23,24). Roberts *et al* reported that SFN binds with plakophilin-3 in desmosomes and decreases incorporation of plakophilin-3 into desmosomes, thereby decreasing desmosomal adhesion and increasing cell migration (27). However, in our study, *TP53* mutated tumors were associated with increased expression of DSC3, DSP, and JUP, which are three genes that encode desmosomal structural proteins, suggesting increased cell adhesion. Genes that were overexpressed in the *TP53*-mutated tumors in our study are often associated with increased adhesion and cell growth inhibition. This means that *TP53*-mutated tumors may have increased mesenchymal-epithelial transition compared to *TP53* wild-type tumors. A difference in the level of mesenchymal-epithelial transition also occurs depending on HPV infection. HPV-positive oropharyngeal squamous cell carcinomas have been reported to lose their epithelial cell phenotype compared with HPV-negative tumors (28). Other studies also have shown that HPV-positive tumors have increased epithelial mesenchymal transition (29,30). HPV-related carcinogenesis is associated with TP53 inactivation, and thus many non-TP53 mutated tumors are HPV positive (13). Therefore, our study findings regarding the expression of genes involved in mesenchymal-epithelial transition in TP53-mutated maxillary carcinoma are consistent with the results of previous studies that showed loss of the epithelial cell phenotype in HPV-positive patients.

TP53 immunostaining in our study showed strong expression in tumor cells regardless of mutation status. Staining was unevenly distributed in *TP53* mutated tumors, with negative staining in the tumor center, but strongly positive staining in the tumor margins. This type of uneven distribution has not previously been reported. This staining differed from that of *TP53* wild-type tumors in which the entire tumor was uniformly stained. In mutated tumors, TP53 degradation may be more likely, or synthesis may be inhibited, in the tumor center. It is also possible that the interaction of tumor cells with the stroma may affect TP53 expression/localization in mutated tumors. This phenomenon whereby tumor cells with different phenotypes are produced may be linked to chemotherapy resistance. Indeed, regression of mutated tumor also occurs with treatment. However, unlike complete regression of wild-type tumors, mutated tumors have some residual treatment-resistant tumor. This may be due to the heterogeneity of *TP53* mutated tumors. Gain-of-function *TP53* mutants have recently been shown to have upregulated chromatin regulatory genes that result in genome-wide increases in histone methylation and acetylation. Knockdown or pharmacological inhibition of these chromatin regulatory genes can markedly lower cancer cell proliferation (31).

Mutated *TP53* can become a new transcription factor leading to transcription activation that does not occur with wild-type *TP53*. Our group has shown that this new transcription activity does actually occur. However, the genes with increased expression in the present study mostly played a role in adhesion and cell growth inhibition. Thus this result suggested that *TP53* mutation in tumors results in a tumor phenotype that is opposite to that of cancer progression and malignant transformation. As represented by SFN, expression of tumor suppressor genes has in fact been observed in chemotherapy-resistant cancer. The significance of this paradoxical phenomenon will require further investigation.

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