Association between *FAT1* mutation and overall survival in patients with human papillomavirus–negative head and neck squamous cell carcinoma

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ABSTRACT: *Background.* The purpose of this study was to characterize the mutation profile of FAT atypical cadherin 1 (*FAT1*) and determine the prognostic significance of *FAT1* mutation for overall survival in patients with human papillomavirus (HPV)-negative head and neck squamous cell carcinoma (HNSCC).

Methods. Data were downloaded from The Cancer Genome Atlas (TCGA) and International Cancer Genome Consortium (ICGC) data portals and used as discovery and validation sets. *FAT1* mutational status was determined in 234 and 37 patients with HPV-negative HNSCC, respectively, and overall survival analysis was performed. For comparison, HPV-positive patients were also analyzed for overall survival.

Results. Most of the identified nonsynonymous somatic *FAT1* mutations were loss-of-function mutations. *FAT1* mutation was significantly associated with better overall survival in HPV-negative patients from both the TCGA cohort ($p = .026$) and the ICGC cohort ($p = .047$), but not in HPV-positive patients.

Conclusion. FAT1 mutational status is a strong independent prognostic factor in patients with HPV-negative HNSCC. *Head Neck* **38**: E2021–E2029, 2016

KEY WORDS: FAT atypical cadherin 1, mutation, human papillomavirus, head and neck neoplasms, prognosis

INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is the seventh most common cancer and the seventh leading cause of cancer-related deaths worldwide, accounting for approximately 650,000 new cases and 350,000 deaths annually.[1] The major risk factors for HNSCC are smoking and alcohol exposure.[2] Recently, human papillomavirus (HPV) has emerged as an additional major risk factor,[3] and HPV-negative and HPV-positive HNSCCs are now considered to represent distinct clinicopathological and biological entities.[4,5] HPV-negative HNSCC, accounting for over 70% of all HNSCCs,[6] is characterized by a predilection for nonoropharyngeal primary sites and frequent genetic alterations induced by smoking and/or alcohol intake, such as *TP53* mutation.[4,5] Furthermore, the response to treatment and the survival are generally worse for patients with HPV-negative HNSCC than for those with HPV-positive HNSCC,[7] making it an important goal to find prognostic markers that could be used for risk stratification and optimization of adjuvant therapy in this subset.

With respect to the survival of patients with HPV-negative HNSCC, it has been established that mutation of the tumor protein p53 (*TP53*) is associated with lower overall survival.[4,8,9] However, with the exception of *TP53*, no marker has been consistently reliable across the plethora of studies assessing the prognostic significance of various candidates; thus, biomarkers of HNSCC prognosis remain elusive.[4]

FAT atypical cadherin 1 (*FAT1*) is a member of the cadherin gene superfamily and it was previously tentatively described as a tumor-suppressor gene.[10,11] More recently, Morris et al[12] reported that *FAT1* is frequently mutated across multiple human cancers, including HNSCC. That study further revealed that loss-of-function mutation of *FAT1* causes Wnt pathway activation and tumorigenesis, and affects patient survival in several human cancers, such as glioma and ovarian cancer, suggesting that *FAT1* is a bona fide tumor-suppressor gene that can drive tumor development in those cancer types. Furthermore, 2 recent

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Additional Supporting Information may be found in the online version of this article.

large-scale exome sequencing studies revealed that *FAT1* is significantly and frequently altered in HNSCCs.[13,14] However, it remains to be determined whether these *FAT1* mutations influence the overall survival of patients with HPV-negative HNSCC.

The purposes of the present study were to characterize the mutation profile of *FAT1* and determine the mutational status of *FAT1* as a prognostic marker for overall survival in surgically treated HPV-negative HNSCC using The Cancer Genome Atlas (TCGA) cohort as a discovery set. For comparison, HPV-positive patients were also analyzed for overall survival, and the prognostic significance of *TP53* was also determined. The results from the TCGA cohort were evaluated further by comparing them with data from a cohort of patients with gingivobuccal squamous cell carcinoma (GBSCC) from the International Cancer Genome Consortium (ICGC) portal. Finally, the relationship between *FAT1* mutation status and mRNA expression level was investigated and the prognostic effect of the *FAT1* expression level was determined in HPV-negative HNSCC.

MATERIALS AND METHODS

Patient data

TCGA patients with HPV-negative HNSCC who underwent surgical resection were used as a discovery set.[13] These publicly available data were downloaded from the TCGA data portal on September 20, 2014. Among 399 patients initially included in the clinical dataset, 320 had HPV-negative tumors. Seven of those patients were excluded because they received neoadjuvant treatment. Whole-exome somatic mutation data were available for 234 of the remaining 313 patients, in whom the *FAT1* mutation profile was analyzed. Among them, clinicopathological data were available for 179 patients without missing values; thus, overall survival analysis was restricted to these patients. For comparison, overall survival analysis was also performed in 38 patients with HPV-positive HNSCC for whom data were available.

The ICGC cohort of patients with HPV-negative GBSCC treated with curative surgery was used as an independent validation set.[14] The clinical dataset released on September 12, 2014, was obtained from the ICGC data portal. Among 50 patients initially included in the clinical dataset, 37 had HPV-negative tumors. None of them received neoadjuvant treatment. Whole-exome somatic mutation data and clinicopathological data were available for all of the 37 patients without missing values; thus, they were all included in this study. For comparison, overall survival analysis was also performed in 13 patients with HPV-positive GBSCC for whom data were available.

Data on human papillomavirus status

In the TCGA cohort, the presence of specific HPV types was detected by using multiplex polymerase chain reaction and mass spectrometry for a panel of 16 HPV types, including HPV16. In the ICGC cohort, the presence of HPV DNA was determined by polymerase chain reaction and Sanger sequencing, and the specific HPV types were determined by Basic Local Alignment Search Tool analysis with the HPV DNA sequences. Detailed information on the HPV detection methods is described elsewhere.[13,14]

Somatic mutation data

For the TCGA cohort, whole-exome somatic mutation data released on October 12, 2012, were downloaded from the TCGA data portal.[13] Whole-exome sequencing was performed using the Illumina Genome Analyzer IIX platform. For the ICGC cohort, whole-exome somatic mutation data released on September 12, 2014, were obtained from the ICGC data portal.[14] Whole-exome sequencing was performed using the Illumina HiSeq 2000 and Roche GS-FLX platforms, and some of the mutations that were sequenced only on one platform were verified using Ion Torrent PGM. Detailed information on the sequencing, quality control, raw data processing, and validation procedure is provided elsewhere.[13,14]

After downloading the data, the somatic mutation profile of *FAT1* and *TP53* was analyzed for each tumor. Nonsense mutations, frameshift indels, and splice-site mutations were considered as loss-of-function mutations. For subsequent analysis, patients were categorized into 2 groups according to their *FAT1* mutational status: those with mutant *FAT1* (ie, presence of at least one nonsynonymous *FAT1* mutation), and those with wild-type *FAT1* (ie, no nonsynonymous *FAT1* mutations).

Bioinformatics analysis

Algorithms for sorting intolerant from tolerant (SIFT) and Polymorphism Phenotyping version 2 (PolyPhen-2) were used to predict the functional effect of the missense *FAT1* mutations detected in the TCGA and ICGC cohorts.[15,16]

RNA-sequencing data

RNA-sequencing (RNA-Seq) data released on March 5, 2014, were downloaded from the TCGA data portal. RNA-Seq by expectation maximization (RSEM) data were generated using the Illumina HiSeq 2000 RNA-Seq version 2 platform, and normalized RSEM data were used to estimate the mRNA expression level.[17]

Statistical analysis

Continuous variables were analyzed using the Mann–Whitney test, and categorical variables were analyzed using Pearson's chi-square test or Fisher's exact test. Survival data were analyzed using the Kaplan–Meier method, and overall survival was compared between the 2 groups using the log-rank test. Multivariate Cox regression analysis was performed to estimate the hazard ratios (HRs) and 95% confidence intervals (95% CIs) for overall survival after adjusting for covariates. Regression diagnostics were performed using Schoenfeld and dfbeta residuals to check the underlying assumptions of the Cox models. Receiver operator characteristic (ROC) curve analysis of the mRNA expression level of *FAT1* was performed to distinguish between tumors with mutant and wild-type *FAT1*. The area under the ROC curve (AUC) was calculated, and its sensitivity and specificity were estimated at the threshold that maximizes "sensitivity + specificity – 1" (hereafter referred to as the Youden-index threshold).[18] All statistical tests were 2-sided, and the threshold for statistical significance was set at $p < .05$, whereas p values between .05 and .10 were considered to indicate marginal

significance. All statistical analyses were performed using R software (version 3.0.2). ROC curve analysis was performed using the pROC package.[19]

RESULTS

Mutation profile of *FAT1* in The Cancer Genome Atlas cohort

In the TCGA cohort, nonsynonymous somatic *FAT1* mutations were identified in 63 of 234 patients (26.9%) with HPV-negative HNSCC. The ratio of nonsynonymous to synonymous mutations was 39.5:1. In total, 79 different nonsynonymous mutations were detected, most of which (77.2%; 61 of 79) were loss-of-function mutations, comprising 37 nonsense mutations, 21 frameshift indels, and 3 splice-site mutations; 17 were missense mutations and 1 was an in-frame deletion (Figure 1A). None of these mutations was tested for validation. The SIFT and PolyPhen-2 algorithms predicted that 13 of 17 (76.5%) missense mutations exerted possible or probable damaging effects on protein function (Supplementary Table S1, online only). Twelve tumors had 2 or 3 mutations, and the overall number of mutations was 1.3 ± 0.1 (mean ± SD). The mutations were evenly distributed throughout the entire gene, with no mutational hotspots (Figure 1B); only 1 mutation (p.Ser2838X) was shared by 2 tumors.

FAT1 mutation frequency was higher in patients with HPV-negative HNSCC (26.9%; 63 of 234) than in those with HPV-positive HNSCC (7.9%; 3 of 38). Each of the 3 HPV-positive patients had one different nonsynonymous mutation, and most of the identified mutations (66.7%; 2 of 3) were loss-of-function mutations. (Supplementary Table S2, online only).

Patient characteristics

The clinicopathological features of the patients with HPV-negative HNSCC in the TCGA cohort are summarized in Table 1. Among the 234 patients, 184 (78.6%) had a history of smoking, 222 (94.9%) had nonoropharyngeal cancer, and 126 (53.8%) had stage IV disease. Most of the patients (79.5%; 186 of 234) had *TP53* mutation. Meanwhile, among the 38 HPV-positive patients, 26 (68.4%) were HPV16 or HPV18 positive (Supplementary Table S3, online only).

Because most of the nonsynonymous mutations in this study were loss-of-function mutations, and the SIFT and PolyPhen-2 algorithms predicted that most of the missense mutations were either possibly or probably damaging, we assumed that most of the nonsynonymous mutations of *FAT1* produced similar deleterious effects on protein function, and so the patients were categorized into those with mutant or wild-type *FAT1*, and the clinicopathological variables were compared between the 2 groups. Patients with mutant *FAT1* were older at diagnosis compared with patients with wild-type *FAT1* (p = .004); however, except for this association, no other significant differences were found between the 2 groups (Table 2).

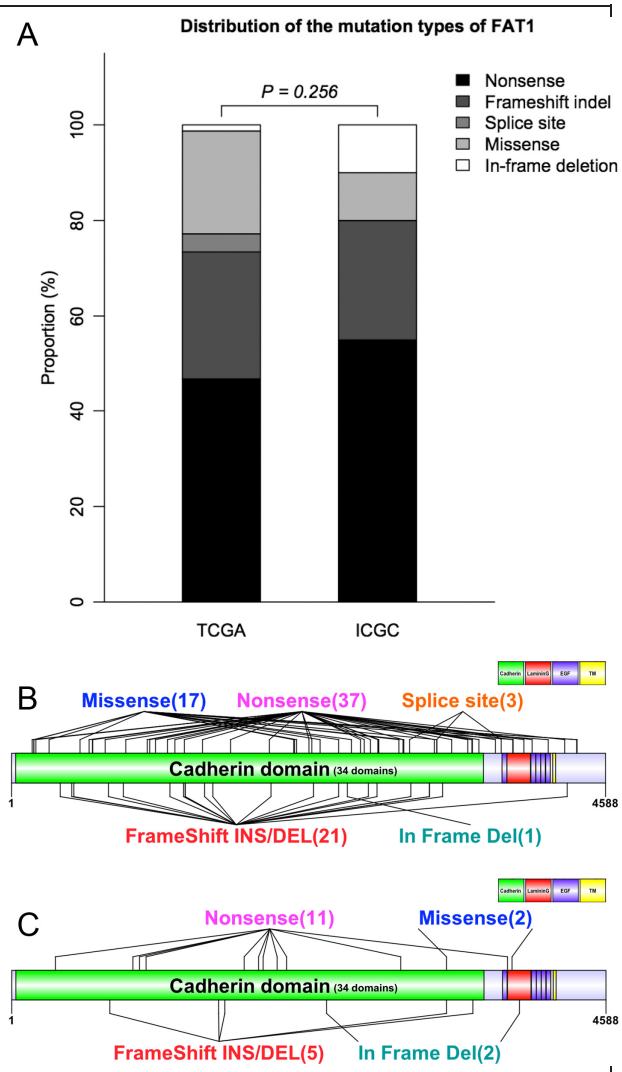

FIGURE 1. Nonsynonymous mutations of FAT atypical cadherin 1 (*FAT1*) in human papillomavirus-negative head and neck squamous cell carcinoma. (A) Distribution of the mutation types of *FAT1* in The Cancer Genome Atlas (TCGA) cohort and the International Cancer Genome Consortium (ICGC) cohort. The distribution did not differ in the 2 cohorts. (B and C) Schematic diagram of the FAT1 protein structure, with the locations of the somatic mutations identified in patients from (B) the TCGA cohort and (C) the ICGC cohort. Boxes represent functional domains, and broken lines indicate the locations of different mutations, which are categorized according to the mutation type. The mutations were distributed throughout the gene with no definite mutational hotspots. Protein domains are annotated using UniProt and the Human Protein Reference Database, and visualized with the aid of Domain Graph (version 2.0).[20]

FAT1 mutational status and overall survival

The median follow-up period among the 179 patients with HPV-negative HNSCC with clinicopathological data was 1.2 years. Univariate Kaplan–Meier analysis revealed that the risk of death was marginally lower for patients with mutant *FAT1* than for those with wild-type *FAT1* (p = .069; Figure 2A), and the risk was significantly higher for patients with mutant *TP53* than for those with

TABLE 1. Clinicopathological characteristics of the patients with human papillomavirus–negative head and neck squamous cell carcinoma.

Variables	TCGA cohort (n = 234)	ICGC cohort (n = 37)
Age, y		
Median	62	46
Range	19–90	26–70
Sex		
Male	160 (68.4%)	35 (94.6%)
Female	74 (31.6%)	2 (5.4%)
Race		
White	200 (85.5%)	0 (0.0%)
Nonwhite	28 (12.0%)	37 (100%)*
Unknown	6 (2.5%)	0 (0.0%)
Smoking history		
Never	40 (17.1%)	2 (5.4%)
Former	107 (45.7%)	35 (94.6%)†
Current	77 (32.9%)	
Unknown	10 (4.3%)	0 (0.0%)
Alcohol history		
Yes	150 (64.1%)	25 (67.6%)
No	80 (34.2%)	12 (32.4%)
Unknown	4 (1.7%)	0 (0.0%)
Primary tumor site		
Oral cavity	154 (65.8%)	37 (100%)‡
Oropharynx	12 (5.1%)	0 (0.0%)
Larynx	68 (29.1%)	0 (0.0%)
Pathological TNM classification		
I	14 (6.0%)	0 (0.0%)
II	37 (15.8%)	2 (5.4%)
III	33 (14.1%)	4 (10.8%)
IV	126 (53.8%)	31 (83.8%)
Unknown	24 (10.3%)	0 (0.0%)
Margin status		
Negative	170 (72.7%)	NA
Close	18 (7.7%)	
Positive	19 (8.1%)	
Unknown	27 (11.5%)	
FAT1 mutation		
Wild-type	171 (73.1%)	16 (43.2%)
Mutant	63 (26.9%)	21 (56.8%)
TP53 mutation		
Wild-type	48 (20.5%)	14 (37.8%)
Mutant	186 (79.5%)	23 (62.2%)

Abbreviations: TCGA, The Cancer Genome Atlas; ICGC, International Cancer Genome Consortium; *FAT1*, FAT atypical cadherin 1; *TP53*, tumor protein p53; NA, not applicable.
* All of the patients were Indian.
† This value includes both current and former smokers.
‡ All of the patients had gingivobuccal squamous cell carcinoma.

wild-type *TP53* (p = .041; Figure 2B). Multivariate Cox proportional hazards regression analysis was performed to adjust for age, sex, race, smoking and alcohol history, primary tumor site, pathological TNM classification, margin status, and *TP53* mutation. This analysis revealed that the risk of death was still significantly lower for patients with mutant *FAT1* than for those with wild-type *FAT1* (HR = 0.511; 95% CI = 0.283–0.921; p = .026; Table 3). In this Cox model, age, pathological TNM classification, and *TP53* mutation were also significant or marginally significant factors for overall survival (p = .004, .073, and .051, respectively).

In the patients with HPV-positive HNSCC, *FAT1* mutation was not associated with overall survival (p = .913; Supplementary Figure S1A, online only). In contrast, *TP53* mutation was significantly associated with shorter overall survival in both univariate and multivariate analysis (p = .010 and .025, respectively; Supplementary Figure S1B, online only, and Supplementary Table S4, online only).

Validation of the prognostic effect of *FAT1* mutation

To validate the prognostic effect of *FAT1* mutation, we used the ICGC cohort of 37 patients with HPV-negative GBSCC as an independent validation set. The patients in this cohort were all Indian and were younger than the patients in the TCGA cohort. Other clinicopathological features of the ICGC patients are summarized in Table 1. Meanwhile, all of the 13 HPV-positive patients were HPV16 and/or HPV18 positive.

FAT1 mutations were identified in 43.2% of the patients (16 of 37) with HPV-negative GBSCC, and the ratio of nonsynonymous to synonymous mutations was 20:1. Most of the identified mutations (80.0%; 16 of 20) were loss-of-function mutations (Figure 1A), and there were no mutational hotspots (Figure 1C). Sixteen of 20 mutations were verified by an orthogonal platform. The *FAT1* mutation profile was compared between the patients from the TCGA and ICGC cohorts. Although no mutations detected in the tumors of the ICGC cohort overlapped with those from the tumors of the TCGA cohort, the distribution of the mutation types and their functional impact on protein was similar in the 2 cohorts (p = .256; Figure 1A and Supplementary Tables S1 and S5, online only). Therefore, the patients in the ICGC cohort were stratified into 2 groups based on their *FAT1* mutational status, as in the TCGA cohort. The distribution of the baseline characteristics did not differ between these 2 groups (Table 2).

The median follow-up period among the 37 patients with HPV-negative GBSCC was 2.0 years. Univariate Kaplan–Meier analysis revealed that mutant *FAT1* was significantly associated with a lower risk of death (p = .034; Figure 2C), whereas *TP53* mutation was not associated with overall survival (p = .983; Figure 2D). The Cox proportional hazards model was used to adjust for other clinicopathological variables; no information was provided regarding margin status, and so it could not be incorporated into the Cox model. This analysis revealed that mutant *FAT1* remained significantly associated with a better overall survival (HR = 0.303; 95% CI = 0.094–0.982; p = .047; Table 3), thus validating the result from the TCGA cohort. Other covariates, including *TP53* mutation (p = .529), were not associated with patient survival. Schoenfeld and dfbeta residual plots revealed that the proportionality assumption was supported for the data (p = .557; Supplementary Figure S2A, online only) and that there were no significant outliers (Supplementary Figure S2B, online only).

Meanwhile, in the patients with HPV-positive GBSCC, the mutational status of neither *FAT1* nor *TP53* was associated with overall survival in both univariate and multivariate analysis (p = .476 and .415 for *FAT1*, and p = .233 and .230 for *TP53*, respectively; Supplementary Figures S1C and S1D, online only, and Supplementary Table S4, online only). Regression diagnostics also revealed that the assumption of proportional hazards held

TABLE 2. Association between the FAT atypical cadherin 1 mutational status and clinicopathological characteristics in patients with human papillomavirus–negative head and neck squamous cell carcinoma.

Variables	TCGA cohort			ICGC cohort		
	Mutant *FAT1* ($n = 63$)	Wild-type *FAT1* ($n = 171$)	*p* value	Mutant *FAT1* ($n = 16$)	Wild-type *FAT1* ($n = 21$)	*p* value
Age, y			.004			.094
Median	64	61		41	50	
Range	28–90	19–87		34–70	26–65	
Sex			.414			.496
Male	40 (63.5%)	120 (70.2%)		16 (100%)	19 (90.5%)	
Female	23 (36.5%)	51 (29.8%)		0 (0.0%)	2 (9.5%)	
Race			.107			1.000
White	52 (82.6%)	148 (86.5%)		0 (0.0%)	0 (0.0%)	
Nonwhite	7 (11.1%)	21 (12.3%)		16 (100%)*	21 (100%)*	
Unknown	4 (6.3%)	2 (1.2%)				
Smoking history			.333			.496
Never	7 (11.1%)	33 (19.3%)		0 (0.0%)	2 (9.5%)	
Former	34 (54.0%)	73 (42.7%)		16 (100%)†	19 (90.5%)†	
Current	19 (30.1%)	58 (34.0%)				
Unknown	3 (4.8%)	7 (4.0%)				
Alcohol history			.478			.491
Yes	39 (61.9%)	111 (65.0%)		12 (75.0%)	13 (61.9%)	
No	24 (38.1%)	56 (32.7%)		4 (25.0%)	8 (38.1%)	
Unknown	0 (0.0%)	4 (2.3%)				
Primary tumor site			.202			1.000
Oral cavity	46 (73.0%)	108 (63.1%)		16 (100%)‡	21 (100%)‡	
Oropharynx	4 (6.4%)	8 (4.7%)		0 (0.0%)	0 (0.0%)	
Larynx	13 (20.6%)	55 (32.2%)		0 (0.0%)	0 (0.0%)	
Pathological TNM classification			.632			1.000
I	3 (4.8%)	11 (6.4%)		0 (0.0%)	0 (0.0%)	
II	14 (22.2%)	23 (13.5%)		1 (6.3%)	1 (4.8%)	
III	8 (12.7%)	25 (14.6%)		2 (12.5%)	2 (9.5%)	
IV	32 (50.8%)	94 (55.0%)		13 (81.2%)	18 (85.7%)	
Unknown	6 (9.5%)	18 (10.5%)				
Margin status			.856			
Negative	45 (71.4%)	125 (73.1%)			NA	
Close	4 (6.4%)	14 (8.2%)				
Positive	5 (7.9%)	14 (8.2%)				
Unknown	9 (14.3%)	18 (10.5%)				
TP53 mutation			.565			.733
Wild-type	15 (23.8%)	33 (19.3%)		7 (43.8%)	7 (33.3%)	
Mutant	48 (76.2%)	138 (80.7%)		9 (56.2%)	14 (66.7%)	

Abbreviations: TCGA, The Cancer Genome Atlas; ICGC, International Cancer Genome Consortium; *FAT1*, FAT atypical cadherin 1; *TP53*, tumor protein p53; NA, not applicable.
* All of the patients were Indian.
† This value includes both current and former smokers.
‡ All of the patients had gingivobuccal squamous cell carcinoma.

true for the data ($p = .756$; Supplementary Figure S2C, online only) and that there were no influential outliers (Supplementary Figure S2D, online only).

We also performed survival analysis for only the nonsense and frameshift *FAT1* mutation in both cohorts. Univariate Kaplan–Meier analysis revealed that *FAT1* mutation was not associated with overall survival in the TCGA cohort ($p = .148$; Supplementary Figure S3A, online only), and marginally associated with better overall survival in the ICGC cohort ($p = .071$; Supplementary Figure S3B, online only). Multivariate analysis revealed that the risk of death was marginally lower for patients with mutant *FAT1* than for those with wild-type *FAT1* in both the TCGA and ICGC cohorts (HR = 0.532; 95% CI = 0.282–1.006; $p = .052$ and HR = 0.353; 95% CI = 0.109–1.146; $p = .083$, respectively; Supplementary Table S6, online only).

Relationship between the mutational status and mRNA expression level of *FAT1*

To investigate the relationship between the mutational status and mRNA expression of *FAT1* in HPV-negative HNSCC, we compared the mRNA expression level of *FAT1* between tumors with mutant versus wild-type *FAT1* using the TCGA RNA-Seq data. The expression of *FAT1* was significantly lower in tumors with mutant *FAT1* ($p < .001$; Figure 3A). ROC curve analysis of the expression level of *FAT1* was performed to distinguish between tumors with mutant versus wild-type *FAT1*, which yielded an AUC of 0.774 (87.3% sensitivity and 60.8% specificity at the Youden-index threshold; Figure 3B).

All patients were divided into high-expression and low-expression groups based on the Youden-index threshold and subjected to Kaplan–Meier analysis to compare the

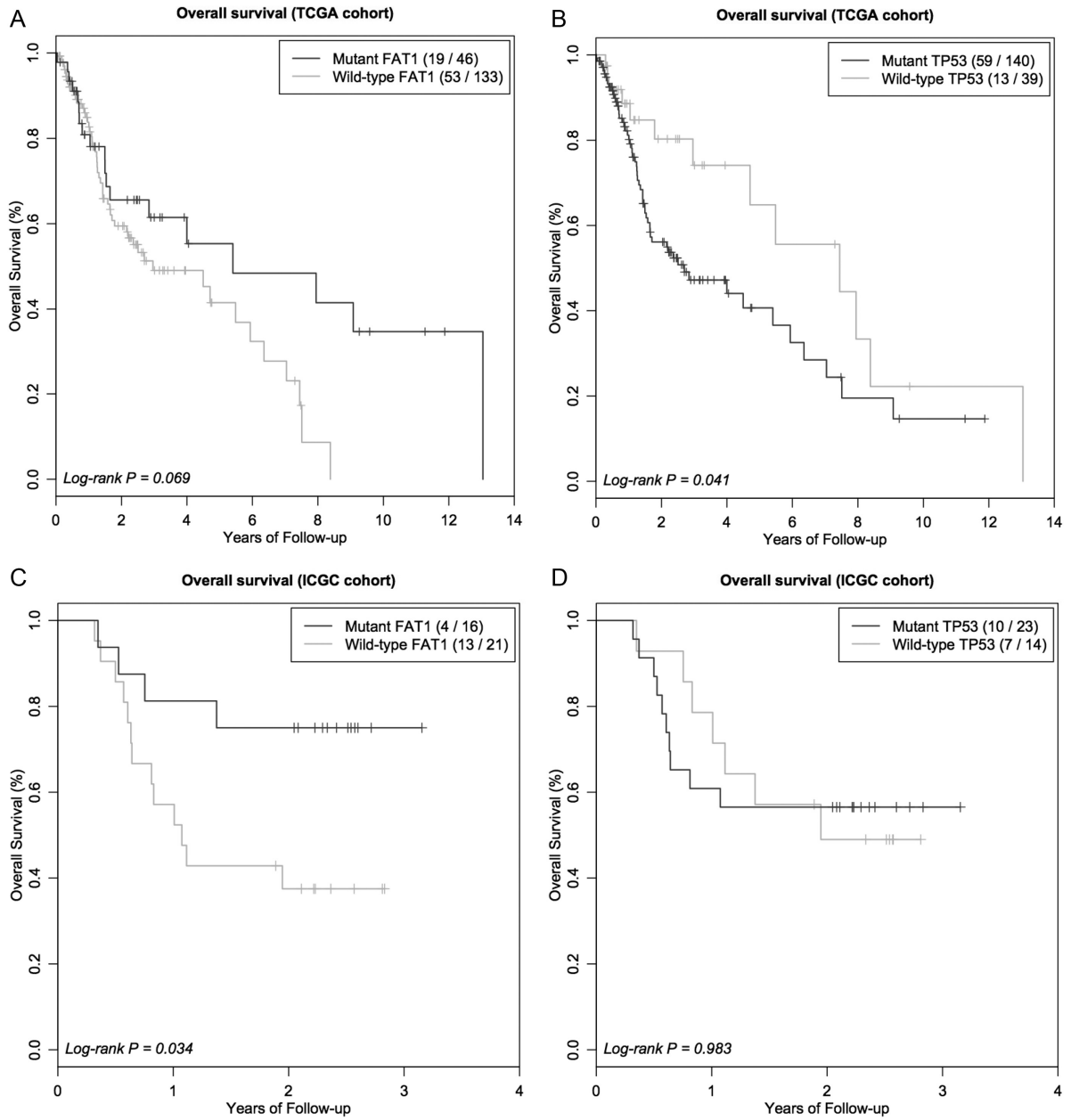

FIGURE 2. Kaplan–Meier survival curves showing survival differences of the patients with human papillomavirus–negative head and neck squamous cell carcinoma according to the mutational status of FAT atypical cadherin 1 (*FAT1*) and tumor protein p53 (*TP53*). In The Cancer Genome Atlas (TCGA) cohort used as a discovery set, (A) overall survival was marginally better for patients with mutant *FAT1* than for patients with wild-type *FAT1*, (B) and *TP53* mutational status was associated with shorter overall survival. Meanwhile, in the International Cancer Genome Consortium (ICGC) cohort used as a validation set, (C) *FAT1* mutation was also associated with longer overall survival, (D) whereas *TP53* mutation was not associated with overall survival.

overall survival between the 2 groups. The expression level of *FAT1* was not associated with overall survival ($p = .312$; Figure 3C).

DISCUSSION

The findings of this study demonstrate that *FAT1* is frequently mutated in HPV-negative HNSCC, most of which were loss-of-function mutations, and that the mutational status of *FAT1* is an independent prognostic factor for overall survival in patients with HPV-negative HNSCC. Nonsynonymous *FAT1* mutation was significantly associated with better overall survival in patients with HPV-negative HNSCC from the TCGA cohort, and the results were validated in patients with HPV-negative GBSCC from the ICGC cohort. Meanwhile, *TP53* mutation was marginally or significantly associated with shorter overall survival in patients with HPV-negative HNSCC from the TCGA cohort, but the results were not validated in the ICGC cohort, probably because of the smallness of the sample. These findings suggest that, at least in the HPV-negative subset, *FAT1* mutation is a stronger prognostic

TABLE 3. Multivariate Cox proportional hazards regression analysis for overall survival in patients with human papillomavirus–negative head and neck squamous cell carcinoma.

Variables	TCGA cohort		ICGC cohort	
	HR (95% CI)	*p* value	HR (95% CI)	*p* value
Age, y	1.040 (1.012–1.069)	.004	0.969 (0.922–1.019)	.222
Sex				
Male vs female	0.954 (0.535–1.703)	.874	0.386 (0.043–3.432)	.393
Race				
White vs nonwhite	0.658 (0.301–1.440)	.295	*	
Smoking history				
Former/current vs never	1.145 (0.500–2.982)	.661	0.994 (0.117–8.449)	.995
Alcohol history				
Yes vs no	0.996 (0.570–1.738)	.988	0.582 (0.180–1.882)	.366
Primary tumor site				
Oral cavity vs oropharynx	0.547 (0.185–1.623)	.277	†	
Larynx vs oropharynx	0.443 (0.135–1.454)	.179	†	
Pathological TNM classification				
IV vs I/II/III	1.746 (0.950–3.210)	.073	1.229 (0.258–5.866)	.796
Margin status				
Positive vs negative	1.234 (0.580–2.623)	.585	NA	
Close vs negative	1.186 (0.510–2.758)	.692		
FAT1 mutation				
Mutant vs wild-type	0.511 (0.283–0.921)	.026	0.303 (0.094–0.982)	.047
TP53 mutation				
Mutant vs wild-type	1.916 (0.997–3.681)	.051	1.426 (0.472–4.306)	.529

Abbreviations: TCGA, The Cancer Genome Atlas; ICGC, International Cancer Genome Consortium; HR, hazard ratio; 95% CI, 95% confidence interval; *FAT1*, FAT atypical cadherin 1; *TP53*, tumor protein p53; NA, not applicable.
* All of the patients were Indian.
† All of the patients had gingivobuccal squamous cell carcinoma.

factor for overall survival than *TP53* mutation. These results also suggest that the mutational status of *FAT1* might be useful in risk stratification and possibly optimization of adjuvant therapy for patients with HPV-negative HNSCC.

FAT1 is one of the human homologues of the *Drosophila fat* gene and belongs to the cadherin gene superfamily.[10] The *fat* gene in *Drosophila* is known to be essential for its developmental processes.[10] With regard to cancer, Bryant et al[11] first reported that lethal mutations of *fat* cause hyperplastic tumor-like overgrowth of larval imaginal discs in *Drosophila*, suggesting that it can act as a tumor-suppressor gene. Since then, there have been several reports of a relationship between genetic alterations of *FAT1* and the tumorigenesis, invasiveness, and/or prognosis of human tumors of various origins, including the oral cavity, brain, breast, and bone marrow.[21–24] More recently, Morris et al[12] showed that the mutation-induced inactivation of *FAT1* promotes Wnt signaling and tumorigenesis in several human cancers and that lower expression of *FAT1* is associated with longer survival in glioma and ovarian cancer. Furthermore, 2 recent large-scale exome sequencing studies reported that *FAT1* is significantly and frequently mutated in HNSCCs.[13,14] However, it was unknown whether the mutational status of *FAT1* is associated with the prognosis in patients with HPV-negative HNSCC. To the best of our knowledge, the present study is the first to show that the nonsynonymous mutational status of *FAT1* is an independent prognostic marker for overall survival in patients with HPV-negative HNSCC.

A few studies, including ours, have shown that inactivation or decreased expression of *FAT1* is associated with longer survival in various tumors,[12,24] but it is as yet unclear how this contributes to a favorable prognosis. The findings of a recent study suggest that the interaction between FAT1 and caspase-8 may represent a mechanism for this protective effect of mutated *FAT1*.[25] In that study, knockdown of FAT1 sensitized primary glioblastoma cells for extrinsic apoptosis by controlling the caspase-8-dependent pathway. In the present study, tumors with mutant *FAT1* also exhibited a significantly lower expression of the gene. Cancer cells can survive and proliferate by deregulating apoptosis, and it has been shown that targeting an extrinsic apoptosis pathway with proapoptotic receptor agonists causes significant regression of tumors.[26] It is therefore likely that inactivation of *FAT1* via mutation, similar to the proapoptotic receptor agonists, suppresses tumor progression through activation of an extrinsic apoptosis pathway.

Agrawal et al[27] reported that the genetic alterations found in HPV-negative HNSCCs are predominantly loss-of-function mutations of tumor-suppressor genes; thus, they cannot be directly targeted by anticancer agents because they are already inactivated, further suggesting that *FAT1* is also not targetable.[28] However, Wang et al[29] reported that simultaneous inactivation of a pair of 2 genes induced by chemical agents can be lethal to cancer cells through a process called "pharmacological synthetic lethality," suggesting that mutated tumor-suppressor genes could be excellent targets for anticancer therapy. Indeed, a recent study showed that FAT1 has a synthetic lethal interaction with death receptor-mediated apoptosis.[25] Furthermore, de Bock et al[24] reported that FAT1 would represent an ideal target for the development of a novel

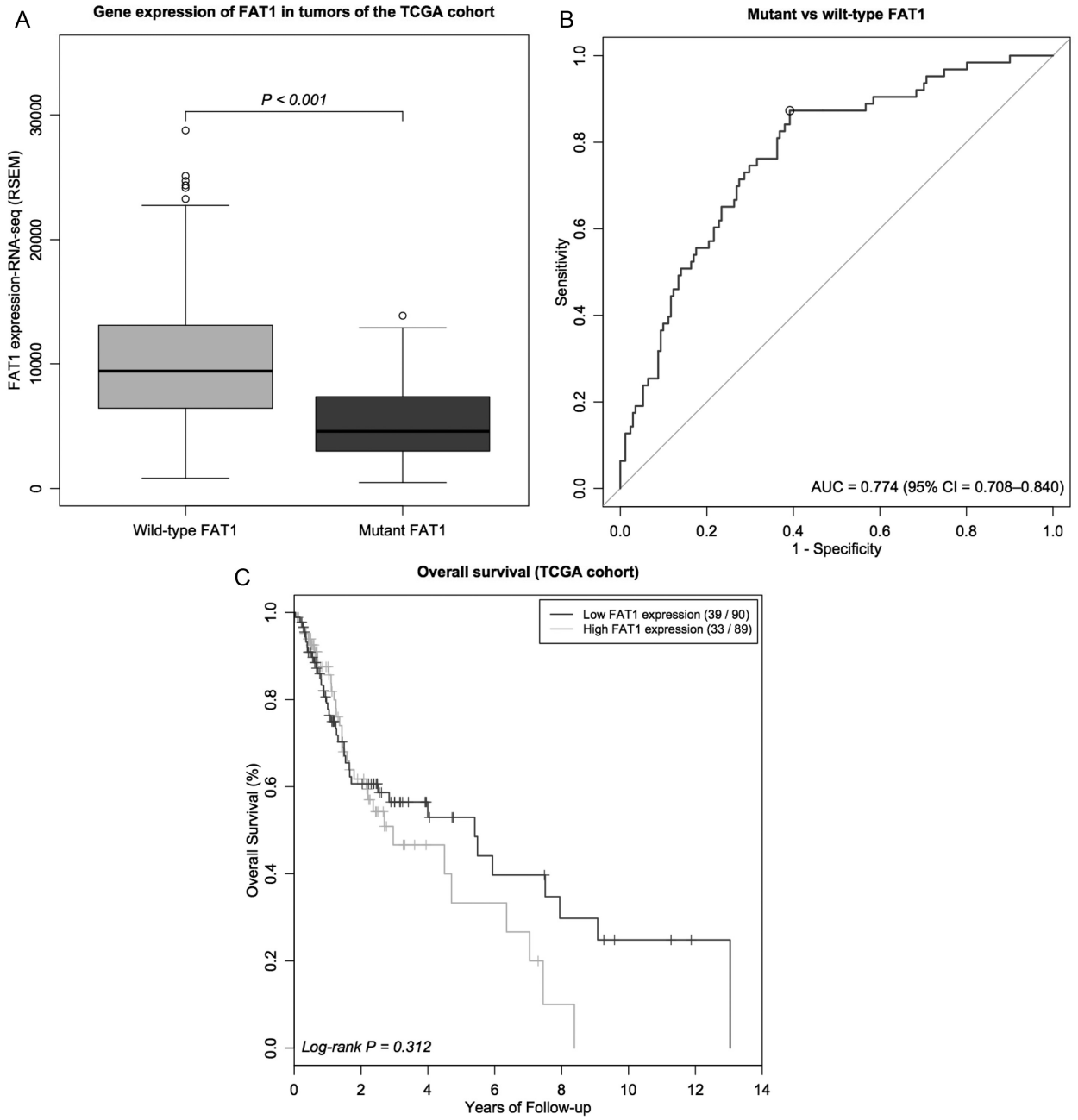

FIGURE 3. RNA-sequencing expression levels of FAT atypical cadherin 1 (*FAT1*) according to its mutational status among patients with human papillomavirus–negative head and neck squamous cell carcinoma from The Cancer Genome Atlas (TCGA) cohort. (A) Tumors with mutant *FAT1* had a significantly lower expression of the gene. (B) A receiver operating characteristic (ROC) curve analysis of *FAT1* expression levels was performed to distinguish between tumors with mutant and wild-type *FAT1*. The open circle on the curve represents the threshold that maximizes the Youden index (hereafter referred to as the Youden-index threshold). (C) Patients were divided into 2 groups on the basis of the Youden-index threshold. Kaplan–Meier analysis revealed that the *FAT1* expression level was not significantly associated with the overall survival. AUC, area under the ROC curve; CI, confidence interval; RSEM, RNA-Seq by expectation maximization.

antibody-based therapeutic agent for leukemias, based on the finding of aberrant *FAT1* mRNA expression in various leukemias, but little or no expression in normal peripheral blood and bone marrow cells. The present study also found that the mRNA expression level differed significantly between tumors with mutant and wild-type *FAT1*. Meanwhile, Liu et al[30] reported that *FAT1* mutation could affect the drug action on Wnt signaling in HNSCC cancer cell lines. Thus, FAT1 may represent a suitable candidate for the development of new cancer therapeutic strategies.

The present findings should be interpreted in the light of certain study limitations. First, the findings of this study were from publicly available data sources: because long-term

follow-up data were not available, median follow-up was not so long, and the smallness of the sample in the validation set might have influenced the result of this study. Second, the ICGC cohort consisted of patients only with GBSCC, a subset of HNSCC, which might have biased the results of this study. It is, of course, likely that the ICGC cohort relatively well reflects the TCGA cohort, first because the incidence rates of *FAT1* mutation were not different between the subsites and stratified Cox regression analysis according to the subsites revealed that *FAT1* mutation was still significantly associated with better overall survival in the TCGA cohort (data not shown), and second because, except for race and primary tumor subsite, baseline characteristics were generally balanced between the TCGA and ICGC cohorts. However, the significance of *FAT1* mutation in other subsites than the gingivobuccal area should be confirmed in the future. Third, many of the *FAT1* mutations detected in this study were not tested for validation, which might have influenced the results of this study. Finally, it was assumed that all of the *FAT1* mutations identified in this study had similar impact on protein function, and the accuracy of this assumption remains to be established.

In conclusion, the findings of this study clearly indicate that the mutational status of *FAT1* is a strong independent prognostic marker for overall survival. We believe that the mutational status of *FAT1* can be potentially useful in risk stratification and optimization of adjuvant therapy after surgery for patients with HPV-negative HNSCC; thus, it could be effective in decreasing mortality because of HPV-negative HNSCC. However, whether the nonsynonymous mutations that occur at different locations along the gene indeed have similar impacts on protein function and patient survival remains to be determined. Moreover, the contribution of *FAT1* mutational status to the survival of patients at the molecular level has yet to be clarified. Further research, including a prospective study with a larger number of cases, is required to confirm the prognostic utility of *FAT1* mutation and to investigate the pathogenetic role of mutated *FAT1* in patients with HPV-negative HNSCC.

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