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Original Article

Prognostic significance of the FSTL1-DIP2A axis in early-stage tongue cancer

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Abstract: In tongue cancer, many patients already have metastasis at the time of diagnosis, and such cases are usually unresponsive to treatment, resulting in a poor prognosis. Therefore, there is an urgent need to develop more effective diagnostic and therapeutic methods to cure tongue cancer at the earliest possible stage in clinical practice. Follistatin-like 1 (FSTL1) is known as a negative effector molecule that induces and enhances the refractoriness of cancer cells directly and indirectly via suppressing anti-tumor immunity in various types of cancer. However, the molecular expression, functions, and clinical significance of FSTL1 and its receptor DIP2A in tongue cancer remains to be elucidated. In this study, we revealed that FSTL1, which is highly expressed in tongue cancer cells, plays a key role in its malignancy and is a significant risk factor for recurrence of early-stage tongue cancer. Basic study shows that FSTL1 is abundantly produced from human tongue cancer cell lines, and blocking FSTL1 with specific siRNAs or mAb significantly suppresses cellular functions. Clinical study shows that both FSTL1 and its receptor DIP2A are highly and correlatively expressed in tumor tissues of tongue cancer patients, and high expression levels of both in stage I tumors are significantly associated with shorter relapse-free survival. These suggest that targeting the FSTL1-DIP2A axis may be useful as a biomarker for early prediction of prognosis in tongue cancer patients, and as a therapeutic target for developing new drugs to treat tongue cancer more effectively. This strategy will contribute to improving clinical outcomes in tongue cancer.

Keywords: Head and neck cancer, oral cancer, tongue cancer, early stage, recurrence, prognosis, proliferation, invasion, FSTL1, DIP2A

Introduction

Tongue cancer is one of the most common sites of head and neck cancer, and accounts for 50-60% of oral cancers [1]. Although oral cancer is most common in people over the age of 55, the incidence of tongue cancer appears to be gradually increasing among young adults [2]. There are few initial symptoms, and thus tumor metastasis is already observed in approximately 25% of patients at the time of diagnosis [1]. Such cases are usually resistant to most treatments, although advanced drugs, including molecular targeted agents such as anti-EGFR mAb [3], and immune checkpoint inhibitory mAbs (ICIs) such as anti-PD1/PDL1 mAbs [4], have been clinically developed to improve the efficacy of the common treatments of surgical tumor resection combined with radiotherapy and chemotherapy. In addition, extended surgery to remove most of the tongue at advanced stages damages the aesthetics and functions using the tongue (tasting, eating, speaking, etc.).

Diagnostic biomarkers for early detection of tongue cancer and accurate prediction of the treatment responses and patient prognosis have been explored in tongue cancer [5]. For example, high PDL1 expression levels in tumor tissues significantly correlate with shorter disease-free survival in tongue cancer patients [6], while there is a contrary report showing a relationship with a favorable prognosis [7]. High tumoral expression levels of EGF, which regulates proliferation of tongue cancer cells, are

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significantly associated with response to the cetuximab treatment [8]. High CCR4 expression levels in tumor tissues are significantly associated with shorter disease-free survival and overall survival (OS) of patients with early-stage pN0 tongue cancer [9]. High plasma levels of CXCL1, which regulates tumor proliferation, migration and invasion of tongue cancer cells, are significantly associated with shorter progression-free survival, OS and early lymphatic metastasis [10]. However, these markers have not yet been applied to practical use. Therefore, there is an urgent need to develop not only biomarkers to accurately predict tongue cancer progression and metastasis as early as possible, but also more effective and less invasive treatments to completely cure tongue cancer.

A member of the SPARC family follistatin-like 1 (FSTL1) has been demonstrated as a key driver of epithelial-to-mesenchymal transition (EMT) and the related cancer stemness of various types of cancers, including hepatocellular carcinoma [11], gastric cancer [12], colorectal cancer [13], breast cancer [14], melanoma [15], and osteosarcoma [16]. A significant correlation between FSTL1 expression levels in tumor tissues and patient prognosis has also been demonstrated in various types of cancers, including esophageal cancer [17], hepatocellular carcinoma [18], colorectal cancer [13, 19, 20], lung cancer [21], and glioblastoma [22]. This suggests that FSTL1 is an important poor prognostic factor in a wide range of cancer types. In tongue cancer, however, the molecular expression, functions, and clinical significance of FSTL1 remain to be elucidated. Therefore, in this study, we analyzed the molecular expression and functions of FSTL1 and its receptor DIP2A using human tongue cancer cell lines in the in vitro assays. In addition, we immunohistochemically analyzed tumor tissues obtained from tongue cancer patients for FSTL1/DIP2A expression, and statistically analyzed the relationship between the molecular expression levels and patient prognosis.

Materials and methods

Tumor cell lines

Human tongue cancer cell lines, SAS and HSC4, were purchased from Cell Resource Center for Biomedical Research at Tohoku University in Japan, and were tested for Mycoplasma negativity using a Hoechst-staining detection kit (MP Biomedicals) before use. The cells were expanded and frozen in liquid nitrogen to avoid changes occurred by the long-term culture. The cells were trypsinized and washed in MEMα (Wako) for assays.

Analysis of tumor cell functions

For knockdown of human *fstl1* gene expression, the specific siRNAs targeting on three different sequence positions of human *fstl1* (NM_007085) were transfected into tumor cells using a Stealth siRNA Set (HSS174138 targeting 507, HSS117328 targeting 563, HSS117329 targeting 701, and the scrambled oligonucleotide as a control; Invitrogen #1299003) after complexed with jetPRIME (PolyPlus) according to the manufacturer's instructions (3 μg, respectively). Before assays, the transfection efficacy was verified by RT-PCR, ELISA, and immunostaining 1-2 days after transfection as described before [21]. Gene expression was assessed by RT-PCR using paired primers specific for human *fstl1* and *gapdh* as described before [21]. For ELISA, cells were cultured in Opti-MEM (GIBCO) for 24 hours, and the supernatant was tested for FSTL1 using a kit (R&D). For immunostaining, smear slides were fixed with 4% PFA and then stained with anti-FSTL1-PE, anti-DIP2A-FITC, and isotype control. For functional analysis, cells were assessed for the proliferative property (3 days) by counting the number of cells, the invasive property (20 hours) using a transwell chamber with a matrigel-coated membrane (Corning), and the adhesive property (3 hours) using a fibronectin-coated 96-well plate (BD Biosciences), as described before [23]. To assess the effect of anti-FSTL1 mAb on cellular functions, tumor cells were cultured in the presence of anti-FSTL1 blocking mAb that we established before [21] or mouse IgG as a control for 4 days, and the cells were tested for cellular functions as described above.

Immunohistochemical analysis

We used tumor tissues archived at the National Cancer Center Biobank in Japan. The tumors were surgically resected from patients with tongue cancer (92% squamous cell carcinoma, 8% verrucous; **Table 1**) at National Cancer Center Hospital (January 1999 -

Table 1. Patient characteristics

Characteristics (n = 53)		
Median age (range)		64 (28-88)
Sex	Male	33 (62%)
	Female	20 (38%)
Pathology	Squamous-Well	39 (74%)
	Squamous-Moderate	1 (2%)
	Squamous-Poor	9 (17%)
	Verrucous	4 (8%)
T stages	Tis	1 (2%)
	I	22 (42%)
	II	20 (38%)
	III	7 (13%)
	IV	3 (6%)
N stages	0	46 (87%)
	I	1 (2%)
	II	6 (11%)
M stages	0	52 (98%)
	I	1 (2%)
Cancer stages	0	1 (2%)
	I	22 (42%)
	II	17 (32%)
	III	7 (13%)
	IVA/IVB/IVC	6 (11%)

December 2006), according to the protocol (No. 2016-067) approved by the Institutional Review Board of the National Cancer Center (n = 53). To exclude the effects of varying treatments, we selected only tumor tissues from patients who had not received any pre-operative treatments. Informed consent was obtained from all individual participants. Immunostaining using the paraffin-embedded tumor sections was conducted according to a standard protocol. Briefly, after deparaffinization, tissue sections were treated with Dako Target retrieval solution (Agilent Technologies) for 5 min, Dako REAL Peroxidase-blocking solution (Agilent Technologies) for 10 min, Dako Protein Block (Agilent Technologies) for 10 min, and the following antibodies as described before [21]: anti-FSTL1-PE that we established before [21], anti-DIP2A-FITC (Bioss), and isotype antibodies as a control (BioLegend) for 24 hours. These tumor sections were also treated with DAPI and haematoxylin as a counterstaining. The immunofluorescence intensity was automatically measured as pixel counts at two fields per section using a LSM700 Laser Scanning Microscope (Carl Zeiss), and the average was used as a data. The mean pixels in tumor tissues stained with isotype control were 579 for FSTL1 and 576 for DIP2A (background). The mean pixels in the normal portion of the tumor tissues were 3,052 for FSTL1 and 5,282 for DIP2A (normal mean). The mean pixels in tumor tissues were 13,311 for FSTL1 and 15,852 for DIP2A (tumor mean). We classified the molecular expression patterns into two types, higher and lower than the tumor mean, and statistically analyzed the relationship between the expression levels and the clinicopathological data of the patients. All activities were conducted in accordance with the ethical principles of the Declaration of Helsinki.

Statistical analysis

Significant differences (P value < 0.05) were statistically evaluated using GraphPad Prism 7 software (MDF) or SPSS Statistics 23.0 software (IBM). Data between two groups were analyzed by the unpaired two-tailed Student's t test. Nonparametric groups were analyzed by the Mann-Whitney test. Correlation between two factors was evaluated by the nonparametric Spearman's rank test. Patient survival was analyzed by the Kaplan-Meier method and the Mantel-Cox Log-Rank test.

Results

FSTL1 knockdown suppresses cellular functions of tongue cancer

We examined the molecular expression and function of FSTL1 using human tongue cancer cell lines, SAS and HSC4. Both cell lines highly expressed and released FSTL1, and these were significantly suppressed by transfection with the *fstl1*-specific siRNAs for FSTL1 knockdown as compared to the control cells transfected with the scrambled oligonucleotide as a control (**Figure 1A**). In the siRNA-*fstl1*-transfected cells, DIP2A expression was simultaneously reduced (**Figure 1A**), and proliferative and inva-

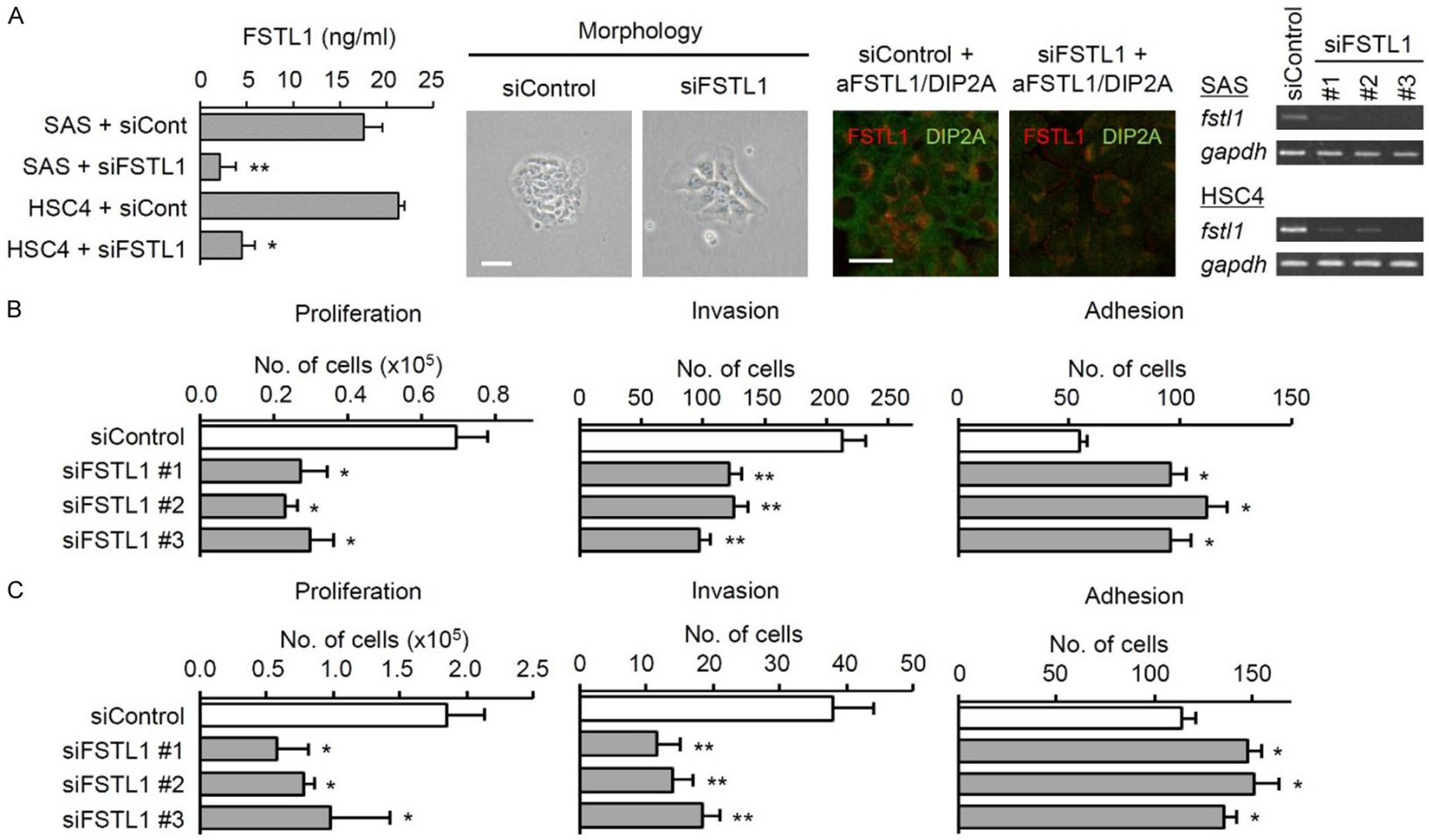

Figure 1. FSTL1 knockdown suppresses cellular functions of tongue cancer. Human tongue cancer cell lines, SAS and HSC4, were transfected with siRNA specific for human *fstl1* gene (siFSTL1#1-3) or the scrambled oligonucleotide as a control (siControl), and 1-2 days later, the cells were tested for *fstl1* gene expression by RT-PCR, FSTL1 production by ELISA, FSTL1 and DIP2A protein expressions by immunostaining, proliferative ability (3 days), invasive ability (20 hours), and adhesive ability (3 hours). A. Suppression of FSTL1 and DIP2A expressions by siRNA-*fstl1* transfection into tongue cancer cells. Representative photos of SAS cells were shown (scale = 50 μm). B. Cellular functions of SAS cells. C. Cellular functions of HSC4 cells. Graphs show means ± SDs (n = 3). *P* values versus siControl-transfected control group by the unpaired two-tailed Student's t test.

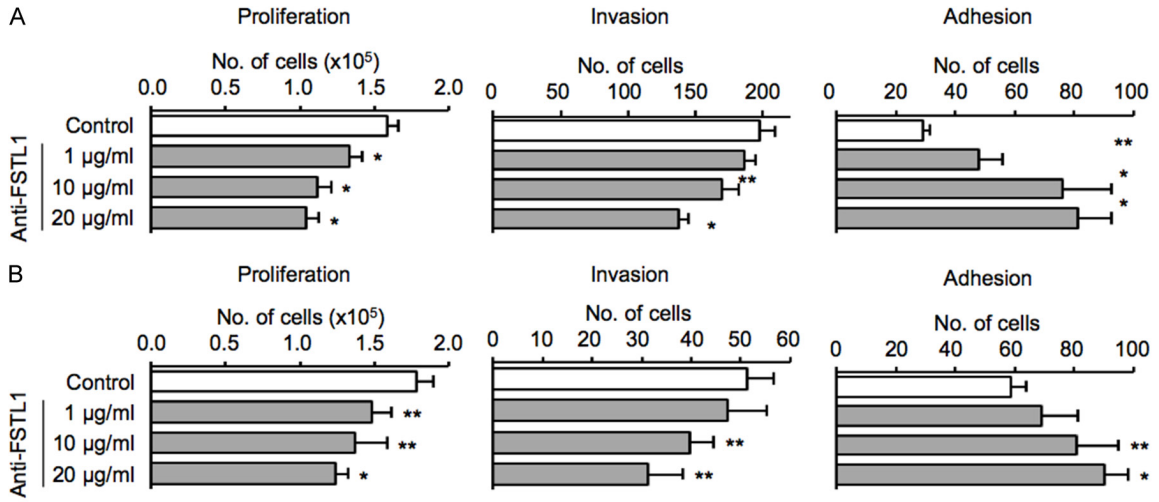

Figure 2. Treatment with anti-FSTL1 blocking mAb suppresses cellular functions of tongue cancer. SAS and HSC4 cells were cultured in the presence of anti-FSTL1 mAb or mouse IgG as a control for 4 days, and were tested for cell proliferation (3 days), cell invasion (20 hours), and cell adhesion (3 hours). A. Cellular functions of SAS cells. B. Cellular functions of HSC4 cells. Graphs show means ± SDs (n = 3). *P* values versus control group by the unpaired two-tailed Student's t test.

sive properties were significantly reduced, but adhesive property was significantly augmented as compared to the control cells ($P < 0.05$; **Figure 1B**, **1C**).

Increased cell proliferation and invasiveness, and decreased adhesion, are typical features of cancer intractability that lead to progression, dissemination, and recurrence [24]. Therefore, these results suggest that FSTL1 is critically involved in malignant properties of tongue cancer cells.

Treatment with anti-FSTL1 blocking mAb suppresses cellular functions of tongue cancer

FSTL1 is a soluble molecule released by tumor cells that acts exogenously to alter their own and surrounding cells. We next examined the effects of the anti-FSTL1 blocking mAb that we previously established [21] on human tongue cancer functions. When SAS and HSC4 cells were cultured in the presence of anti-FSTL1 blocking mAb, the proliferative and invasive properties were significantly reduced, but adhesive property was significantly enhanced as compared to the control treated with mouse IgG as a control, although the effects were lower than those of siRNA transfection ($P < 0.05$; **Figure 2**). These suggest that tongue cancer cellular functions can be suppressed not only by silencing endogenous FSTL1 expression/production, but also by inhibiting FSTL1 that is produced extracellularly.

Significant correlation between FSTL1 and DIP2A expression in tongue tumors of patients

Then, we immunohistochemically analyzed tumor tissues obtained from tongue cancer patients for FSTL1 and DIP2A expressions (n = 53; **Table 1**). We collected tumor specimens from tongue cancer patients who had not received any pre-operative treatments in order to exclude the effects of drug treatment, which is relatively unstandardized and varies in tongue cancer. The expression levels of both FSTL1 ($P = 0.008$) and DIP2A ($P = 0.001$) were significantly increased in tumor tissues compared to normal tissues, and a significant correlation between both expressions was observed ($P < 0.001$; **Figure 3**). There were no significant differences in both expressions between each stage: the mean FSTL1 expression was 9,487 in stage 0/I (n = 23), 19,362 in stage II (n = 17), 13,283 in stage III (n = 7), and 10,855 in stage IV (n = 6); and the mean DIP2A expression was 13,867 in stage 0/I, 15,761 in stage II, 23,800 in stage III, and 14,448 in stage IV. This suggests that both FSTL1 and DIP2A are widely involved in all pathogenic stages of tongue cancer.

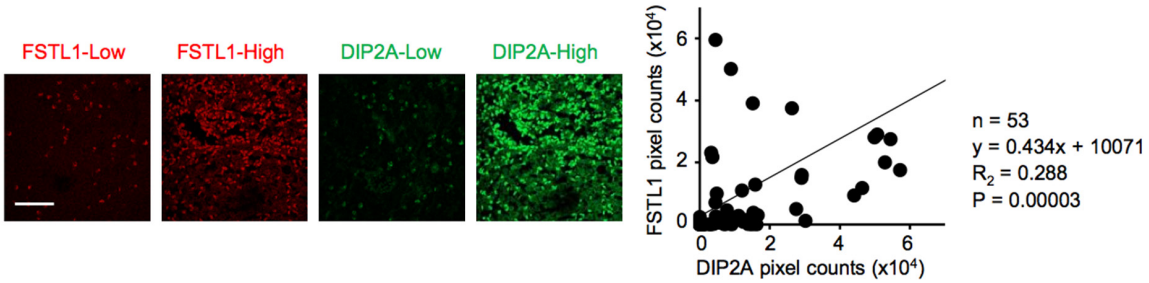

Figure 3. Significant correlation between FSTL1 and DIP2A expression in tongue tumors of patients. Tumor tissues obtained from tongue cancer patients (n = 53) were stained with anti-FSTL1-PE and anti-DIP2A-FITC, and the immunofluorescence intensity was automatically measured as pixel counts at two fields per section. The average was used in the dot graph. The mean pixels stained with isotype control as a background were 579 for FSTL1 and 576 for DIP2A. The mean pixels in normal tissues were 3,052 for FSTL1 and 5,282 for DIP2A. The mean pixels in tumor tissues were 13,311 for FSTL1 and 15,852 for DIP2A. Representative photos are shown (scale = 50 μm). *P* value by the nonparametric Spearman's rank test.

High expression levels of FSTL1 and DIP2A tend to be a risk factor for recurrence in tongue cancer

Patients were divided into two groups, higher and lower molecular expression levels, based on the mean pixel value in the tumor tissues, and the relationship with the clinicopathological data was statistically analyzed. Although no significant differences were observed in relation to the other clinicopathological data, patients with high FSTL1 levels showed a slight trend toward shorter recurrence-free survival (RFS) as compared to patients with low levels (P = 0.30, hazard ratio [HR] = 1.51; **Figure 4A**). As well, patients with high DIP2A levels showed a tendency for both RFS (P = 0.09, HR = 1.96) and OS (P = 0.16, HR = 1.82) to be shorter as compared to patients with low levels (**Figure 4B**). Even when analyzing FSTL1 and DIP2A in combination, however, no significant difference in PFS (P = 0.10, HR = 2.03) and OS (P = 0.22, HR = 1.76) was observed between the FSTL1/DIP2A-high group and the other group (**Figure 4C**). These suggest that high expression levels of either FSTL1 or DIP2A appear to be less relevant to poor prognosis in patients with tongue cancer.

The combination of the high FSTL1/DIP2A expression levels is a significant risk factor for recurrence in early-stage tongue cancer

We next analyzed the data separately for each stage, and found that high expression levels of FSTL1 (P = 0.06, HR = 3.27; **Figure 5A**) and DIP2A (P = 0.18, HR = 2.39; **Figure 5B**) was more clearly associated with shorter RFS only in patients with stage I tumors, albeit no significance. When FSTL1 and DIP2A were analyzed in combination, patients with high FSTL1/DIP2A levels showed significantly shorter RFS as compared to the other patients (P = 0.04, HR = 5.71; **Figure 5C**). The HR was strikingly increased by the combination, and 80% of patients without elevated expression of either were relapse-free over a 10-year period. Patients with high FSTL1/DIP2A levels also showed a tendency to be shorter OS as compared to the other patients, albeit no significance (P = 0.14, HR = 2.93; **Figure 5C**). These suggest that the high FSTL1/DIP2A expression levels are a significant risk factor for recurrence in early-stage tongue cancer. Taken together, targeting the FSTL1/DIP2A axis may be a promising strategy to improve clinical outcomes in tongue cancer.

Discussion

Despite advances in diagnosis and treatment strategies due to the identification of various key factors in tongue cancer, no reliable one has yet been established in clinical practice. In this study, we provide a potential rationale for targeting them in diagnosis and treatment of tongue cancer. Both FSTL1 and its receptor DIP2A were highly and correlatively expressed in tumor cells and tumor tissues obtained from tongue cancer patients, and the high expression levels of both in stage I tumors were significant risk factors for recurrence in tongue cancer. FSTL1 blockade with the specific siRNAs and mAb significantly suppressed cellular

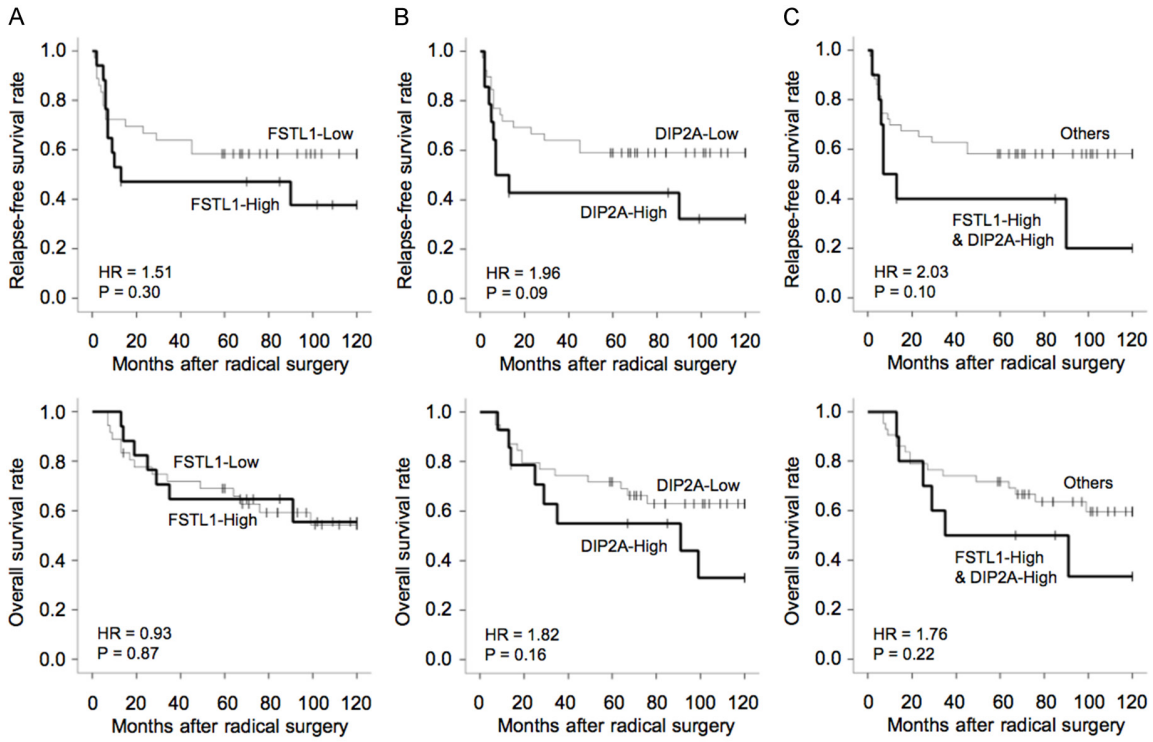

Figure 4. Impact of FSTL1 and DIP2A expressions on prognosis of tongue cancer. Patients with tongue cancer were divided into two groups, high (bold lines) and low (thin lines), based on the mean pixels in tumor tissues (13,311 for FSTL1, and 15,852 for DIP2A), and the relationship between expression levels and patient prognosis, relapse-free survival (RFS; upper) and overall survival (OS; lower), was statistically analyzed by the Kaplan-Meier method and the Mantel-Cox Log-Rank test. A. Relationship with FSTL1 expression. High group, n = 17. Low group, n = 36. B. Relationship with DIP2A expression. High group, n = 14. Low group, n = 39. C. Relationship with the combined FSTL1/DIP2A expression. FSTL1/DIP2A high group, n = 10. Other group, n = 43.

functions as well as DIP2A expression of human tongue cancer cells. These suggest that FSTL1 plays a key role in malignant properties of tongue cancer. Considering that FSTL1 is involved in the EMT mechanisms that control cancer stemness, it is speculated that increased FSTL1/DIP2A expression in tongue cancer at early stages would have a significant impact on the subsequent status of tumors and treatment efficacy in the patients. Thus, targeting the FSTL1-DIP2A axis may be a promising strategy for improving clinical outcomes in tongue cancer.

In tongue cancer, many patients already have metastasis at the time of diagnosis, and such metastatic cases are usually unresponsive to treatments [1]. Patients who reach the advanced stages may lose their tongue through extended surgery to remove most of the tongue, and this may damage the aesthetics, various functions using the tongue, and quality of life of the patients. FSTL1 may be a good target molecule for biomarkers that can more accurately predict and diagnose the onset, progression, and prognosis of tongue cancer in order to treat it as early as possible, and also for therapeutics that can treat tongue cancer more effectively and less invasively.

Accumulating evidence suggests that epigenetic modification by host environment, which is composed of numerous components including stromal cells, vascular cells and immune cells, impacts on every step of the tumor progression process (development, proliferation, dissemination, invasion, intravasation, extravasation, colonization, survival, etc.), and the intrinsic changes in tumor cells are fostered by numerous components in the host [25, 26]. Enormous heterogeneity and complexity of the oncoimmunological network are further produced by the interplay between tumor cells and host immunity in cancer patients, and the recip-

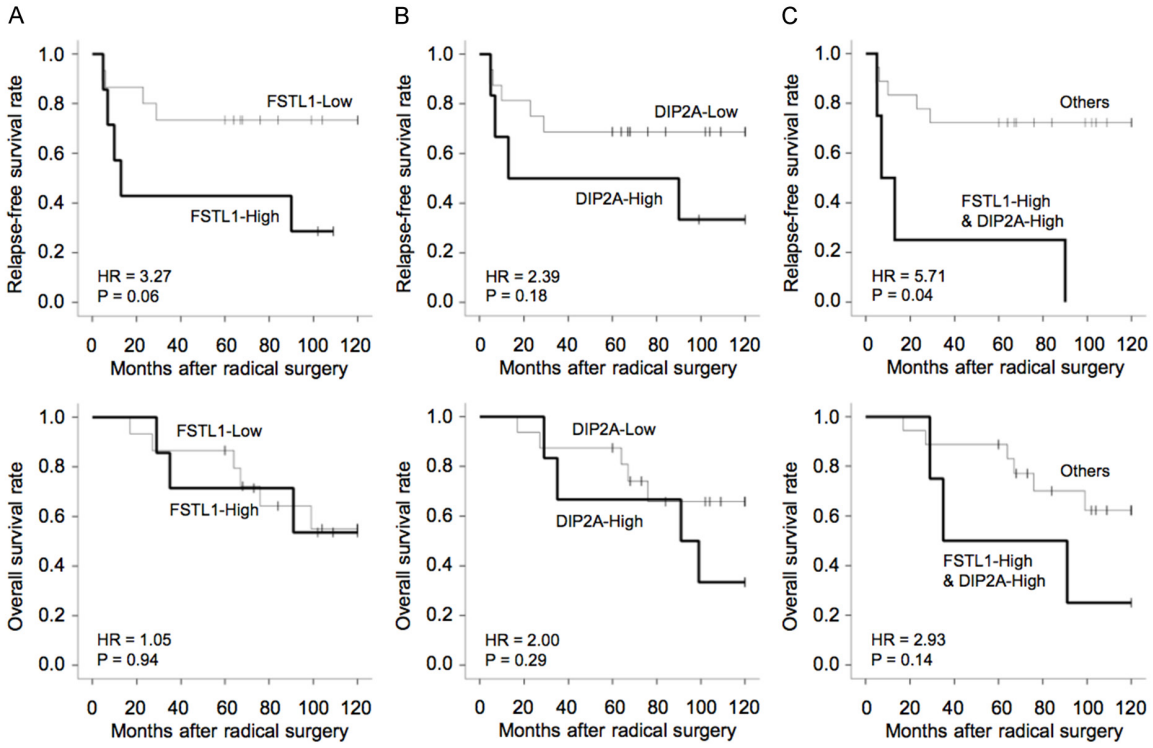

Figure 5. High FSTL1/DIP2A expressions are a significant risk factor for recurrence in early-stage tongue cancer. Patients with stage I tongue cancer (n = 22) were divided into two groups, high and low, based on the mean pixels in tumor tissues, and the relationship between expression levels and patient prognosis, RFS and OS, was statistically analyzed Kaplan-Meier method and the Mantel-Cox Log-Rank test. A. Relationship with FSTL1 expression. High group, n = 7. Low group, n = 15. B. Relationship with DIP2A expression. High group, n = 6. Low group, n = 16. C. Relationship with the combined FSTL1/DIP2A expression. FSTL1/DIP2A high group, n = 4. Other group, n = 18.

rocal evolution among these factors consequently generates refractory cancer [25, 26]. These suggest that it is also important to understand the immunological roles of FSTL1 and DIP2A as well as biological roles in tongue cancer. For that, further studies using human samples (peripheral blood cells, tumor-infiltrating cells, etc.) and mouse tongue cancer models are needed, although these materials were not available to us at this time.

As we previously reported in other cancer [13, 16], FSTL1 expression was closely linked to DIP2A expression in tongue cancer, since both expressions were significantly correlated in tongue cancer tissues of patients, and FSTL1 knockdown in tongue cancer cells simultaneously decreased DIP2A expression. DIP2A expression may be induced and maintained in response to the released FSTL1 in an autocrine manner, thereby maintaining and fostering the intractability of cancer as previously reported in other cancers [13, 16]. FSTL1 and DIP2A expressions were increased in tumor tissues at all stages, but were correlated only with stage I prognosis, implying that FSTL1 might play a key role in tongue cancer progression and metastasis, especially at the onset of developing into a refractory phenotype potentially via EMT. Collectively, targeting the FSTL1/DIP2A axis will contribute to improving the clinical outcomes in tongue cancer.

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Disclosure of conflict of interest

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References

[1] Gonzalez M and Riera March A. Tongue cancer. StatPearls. Treasure Island (FL) ineligible companies. Disclosure: Antonio Riera March declares no relevant financial relationships with ineligible companies. 2024.

[2] Paderno A, Morello R and Piazza C. Tongue carcinoma in young adults: a review of the literature. Acta Otorhinolaryngol Ital 2018; 38: 175-180.

[3] Al-Saleh K, El-Sherify M, Safwat R, Elbasmy A, Shete J, Hussein A, Nazeeh M and Bedair A. Phase II/III randomized controlled trial of concomitant hyperfractionated radiotherapy plus cetuximab (Anti-EGFR Antibody) or chemotherapy in locally advanced head and neck cancer. Gulf J Oncolog 2019; 1: 6-12.

[4] Kujan O, van Schaijik B and Farah CS. Immune checkpoint inhibitors in oral cavity squamous cell carcinoma and oral potentially malignant disorders: a systematic review. Cancers (Basel) 2020; 12: 1937.

[5] Zheng M, Li L, Tang YL and Liang XH. Biomarkers in tongue cancer: understanding the molecular basis and their clinical implications. Postgrad Med J 2010; 86: 292-298.

[6] Akisada N, Nishimoto K, Takao S, Gion Y, Marunaka H, Tachibana T, Makino T, Miki K, Akagi Y, Tsumura M, Toji T, Yoshino T, Nishizaki K, Orita Y and Sato Y. PD-L1 expression in tongue squamous cell carcinoma. Med Mol Morphol 2021; 54: 52-59.

[7] Yoshida S, Nagatsuka H, Nakano K, Kogashiwa Y, Ebihara Y, Yano M and Yasuda M. Significance of PD-L1 expression in tongue cancer development. Int J Med Sci 2018; 15: 1723-1730.

[8] Ansell A, Jedlinski A, Johansson AC and Roberg K. Epidermal growth factor is a potential biomarker for poor cetuximab response in tongue cancer cells. J Oral Pathol Med 2016; 45: 9-16.

[9] Wang L, Zhang M, Zhu Y, Zhang X, Yang Y and Wang C. CCR4 expression is associated with poor prognosis in patients with early stage (pN0) oral tongue cancer. J Oral Maxillofac Surg 2019; 77: 426-432.

[10] Zhang S, Dong Y, Zhao S, Bi F, Xuan M, Zhu G, Guo W and Zhang Z. CXCL1 promoted the migration and invasion abilities of oral cancer cells and might serve as a promising marker of prognosis in tongue cancer. J Oral Pathol Med 2023; 52: 583-592.

[11] Loh JJ, Li TW, Zhou L, Wong TL, Liu X, Ma VWS, Lo CM, Man K, Lee TK, Ning W, Tong M and Ma S. FSTL1 secreted by activated fibroblasts promotes hepatocellular carcinoma metastasis and stemness. Cancer Res 2021; 81: 5692-5705.

[12] Wu M, Ding Y, Wu N, Jiang J, Huang Y, Zhang F, Wang H, Zhou Q, Yang Y, Zhuo W and Teng L. FSTL1 promotes growth and metastasis in gastric cancer by activating AKT related pathway and predicts poor survival. Am J Cancer Res 2021; 11: 712-728.

[13] Kudo-Saito C, Ogiwara Y, Imazeki H, Boku N, Uemura Y, Zhang R, Kawano-Nagatsuma A, Kojima M and Ochiai A. CD11b(+)DIP2A(+) LAG3(+) cells facilitate immune dysfunction in colorectal cancer. Am J Cancer Res 2021; 11: 5428-5439.

[14] Cheng S, Huang Y, Lou C, He Y, Zhang Y and Zhang Q. FSTL1 enhances chemoresistance and maintains stemness in breast cancer cells via integrin beta3/Wnt signaling under miR-137 regulation. Cancer Biol Ther 2019; 20: 328-337.

[15] Kudo-Saito C, Fuwa T, Murakami K and Kawakami Y. Targeting FSTL1 prevents tumor bone metastasis and consequent immune dysfunction. Cancer Res 2013; 73: 6185-6193.

[16] Ogiwara Y, Nakagawa M, Nakatani F, Uemura Y, Zhang R and Kudo-Saito C. Blocking FSTL1 boosts NK immunity in treatment of osteosarcoma. Cancer Lett 2022; 537: 215690.

[17] Lau MC, Ng KY, Wong TL, Tong M, Lee TK, Ming XY, Law S, Lee NP, Cheung AL, Qin YR, Chan KW, Ning W, Guan XY and Ma S. FSTL1 promotes metastasis and chemoresistance in esophageal squamous cell carcinoma through NFkappaB-BMP signaling cross-talk. Cancer Res 2017; 77: 5886-5899.

[18] Yang W, Wu Y, Wang C, Liu Z, Xu M and Zheng X. FSTL1 contributes to tumor progression via attenuating apoptosis in a AKT/GSK-3beta - dependent manner in hepatocellular carcinoma. Cancer Biomark 2017; 20: 75-85.

[19] Gu C, Wang X, Long T, Wang X, Zhong Y, Ma Y, Hu Z and Li Z. FSTL1 interacts with VIM and promotes colorectal cancer metastasis via activating the focal adhesion signalling pathway. Cell Death Dis 2018; 9: 654.

[20] Zhao Y, Ou Q, Deng Y, Peng J, Li C, Li J, Zhao Q, Qiu M, Wan D, Fang Y and Pan Z. Determination of follistatin-like protein 1 expression in colorectal cancer and its association with clinical outcomes. Ann Transl Med 2019; 7: 606.
[21] Kudo-Saito C, Ishida A, Shouya Y, Teramoto K, Igarashi T, Kon R, Saito K, Awada C, Ogiwara Y and Toyoura M. Blocking the FSTL1-DIP2A axis improves anti-tumor immunity. Cell Rep 2018; 24: 1790-1801.
[22] Nie E, Miao F, Jin X, Wu W, Zhou X, Zeng A, Yu T, Zhi T, Shi Z, Wang Y, Zhang J, Liu N and You Y. Fstl1/DIP2A/MGMT signaling pathway plays important roles in temozolomide resistance in glioblastoma. Oncogene 2019; 38: 2706-2721.
[23] Kudo-Saito C, Shirako H, Takeuchi T and Kawakami Y. Cancer metastasis is accelerated through immunosuppression during Snail-induced EMT of cancer cells. Cancer Cell 2009; 15: 195-206.
[24] Ribatti D, Tamma R and Annese T. Epithelial-mesenchymal transition in cancer: a historical overview. Transl Oncol 2020; 13: 100773.
[25] Marzagalli M, Ebelt ND and Manuel ER. Unraveling the crosstalk between melanoma and immune cells in the tumor microenvironment. Semin Cancer Biol 2019; 59: 236-250.
[26] van Weverwijk A and de Visser KE. Mechanisms driving the immunoregulatory function of cancer cells. Nat Rev Cancer 2023; 23: 193-215.