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Expression of interleukin-33 is correlated with poor prognosis of patients with squamous cell carcinoma of the tongue

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ABSTRACT

Objective: The aim of this study was to clarify the role of IL-33 in tumor progression.
Methods: Surgical specimens from 81 patients with squamous cell carcinoma of the tongue were studied using immunohistochemistry. Primary tumor sections were analyzed for IL-33 and ST2 expression. To examine the influence of IL-33 on the microenvironment of the tumor, we determined the mast cell density (MCD) and microvessel density of the stroma.
Results: Patients with high IL-33 expression had a significantly worse prognosis ($p = 0.004$). IL-33 expression was significantly elevated in patients with local and nodal recurrence ($p = 0.014$ and $p = 0.019$). ST2 expression was also associated with a worse prognosis ($p = 0.024$) and was significantly elevated in patients with nodal recurrence ($p = 0.004$). MCD was associated with worse prognosis ($p = 0.038$) and correlated significantly with IL-33 expression ($r = 0.626$, $p < 0.001$). Micovessels in the stroma were significantly increased in the high IL-33 group ($p < 0.001$).
Conclusion: These data suggest that the IL-33/ST2 axis contributes to tumor aggressiveness and affects the tumor microenvironment. Immunohistochemical evaluation of IL-33 and ST2 is useful for identifying patients at a high risk for poor prognosis.

1. Introduction

Tongue squamous cell carcinoma (SCC) is one of the most common head and neck cancers. Prognosis is associated with clinical stage, particularly nodal status. Therefore, the expression of metastasis-related factors such as matrix metalloproteinases, syndecans, and vascular endothelial growth factor predicts the treatment outcome of patients with tongue SCC [1–4]. Tongue SCC is associated with inflammation in the oral cavity, which is mediated by chronic trauma such as smoking, alcohol consumption, and periodontal disease and is associated with oral carcinogenesis [5]. Moreover, inflammation promotes cancer development and progression through its effects on the tumor microenvironment [6]. Inflammatory mediators and specific cell types influence the migration, invasion, and metastasis of tumor cells [6]. Therefore, efficacious therapies must be developed for targeting inflammation in patients with cancer.

Interleukin (IL)-33 is a member of the IL-1 family and functions as a ligand for ST2, which is a member of the IL-1 receptor family. IL-33 is expressed by many cell types, including epithelial cells, endothelial cells, smooth muscle cells, fibroblasts, and activated macrophages [7]. In contrast, mast cells (MCs), Th2 cells, eosinophils, basophils, epithelial cells, and endothelial cells express ST2 [8–12]. Unlike other members of the IL-1 family, the cleavage site for caspases is located within the IL-1-like domain of IL-33, and the cleavage products are biologically inactive.

Biologically active, full-length IL-33 is released when cells sense inflammatory signals or undergo necrosis. Therefore, IL-33 acts as an endogenous danger signal or "alarmin" [8,13,14]. Binding of extracellular IL-33 to ST2 preferentially induces Th2-type immune responses with concomitant expression of Th2-associated cytokines [7]. It is clear that the potency of IL-33 for activating several immunocytes probably impacts inflammation. For example, IL-33 is expressed by patients with chronic gastritis, chronic hepatitis, and inflammatory bowel disease [15–17]. Inflammatory diseases increase the risk of developing cancer, suggesting that IL-33 may play an important role in cancer pathogenesis [18,19].

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IL-33 affects the phenotype of cell types that express the transmembrane isoform of ST2. The inflammatory component of a neoplasm may include a diverse leukocyte population, and ST2 is expressed on many of these inflammatory cells, including MCs, macrophages, dendritic cells, eosinophils, and neutrophils [8,20]. Among the inflammatory cells in the stroma of the tumor, MCs are important because they secrete cytokines and chemokines, and influence the phenotypes of other cells through these soluble mediators as well as through cell–cell interaction. Moreover, MCs reside in the connective tissue surrounding tumors, and the accumulation of MCs is often associated with poor prognosis [21,22]. IL-33 is a potent activator of MCs and induces their degranulation and maturation, promotes survival, and induces the production of several proinflammatory cytokines [9,23]. Therefore, it is reasonable to assume that IL-33 contributes significantly to the malignant potential of tumor cells through the formation of an inflammatory tumor microenvironment.

We reported the carcinogenic role of activation-induced cytidine deaminase (AID), which is induced by an inflammatory environment [24]. However, the expression of AID does not correlate with the progression of tongue SCC, which diverted our attention to IL-33. Inflammation mediated by tobacco smoking, which is associated with tongue SCC, induces the expression of IL-33/ST2 in mice [25]. In the present study, we determined the expression of IL-33 in patients with tongue SCC using immunohistochemistry and assessed the relationship between the expression of IL-33 and prognosis of tongue SCC. Furthermore, to evaluate the influence of IL-33 on the tumor microenvironment, we determined the density of MCs in the stroma surrounding the tumor.

2. Materials and methods

2.1. Patients and specimens

This study included 81 patients who were diagnosed with tongue SCC. Informed consent was obtained from all patients in accordance with our institutional guidelines. The patient characteristics are presented in Table 1. Their clinical status was determined according to the TNM classification system of the Union Internationale Contre le Cancer [26]. All patients underwent surgery at the Division of Otolaryngology-Head and Neck Surgery, Kanazawa University Hospital between 1982 and 2007. Resection of the primary tongue tumor was performed in all patients, and neck dissection was performed in 53 patients with clinically positive nodes or tumors that were more advanced than stage T2. The 22 patients with positive margins or pathologically positive lymph node metastasis underwent postoperative treatment, including radiotherapy. The mean follow-up period was 50.7 months (median, 41 months; range, 1–131 months). Disease-free survival was calculated from the date of treatment until the time of local recurrence or the detection of metastases, including recurrence in the neck lymph nodes.

2.2. Immunohistochemical analysis

All specimens were the primary tumors. They were fixed in 10% formalin solution and embedded in paraffin. Serial 3-μm-thick sections were cut from each block, dewaxed, and rehydrated. Antigen retrieval was performed by heating slides for 30 min in citrate buffer (pH 6.0) at 90 °C, cooling for 20 min, and washing. Endogenous peroxidase was quenched with methanol and 3% H_2O_2 for 10 min, followed by incubation with Protein Block Serum (DakoCytomation, Glostrup, Denmark) to decrease nonspecific binding. Then, the sections were incubated overnight at 4 °C with primary antibodies against IL-33 (diluted 1:100, rabbit polyclonal, Medical & Biological Laboratories, Nagoya, Japan), ST2 (diluted 1:100, mouse monoclonal, Medical & Biological Laboratories), mast cell tryptase (diluted 1:1000, mouse monoclonal, Dako), and CD34 (diluted 1:50, mouse monoclonal, Dako). The sections were incubated with secondary antibodies conjugated to a peroxidase-labeled polymer (EnVision™+ system, Dako) at room temperature for 30 min. Immune complexes were detected using 3,3′-diaminobenzidine tetrahydrochloride, and the sections were counterstained with hematoxylin. The specificities of the staining reactions were confirmed using nonimmune serum instead of the primary antibody.

Table 1
Relationship between clinicopathological features and expression of IL-33, ST2, and MCD.

Characteristic		Samples	Number of high IL-33 expression (%)	*p*	Number of high ST2 expression (%)	*p*	Number of high MCD (%)	*p*
Sex	Male	50	24 (48.0)	0.550	25 (50.0)	0.888	25 (50.0)	0.888
	Female	31	17 (54.8)		16 (51.6)		16 (51.6)	
Age	≦60	40	17 (42.5)	0.149	20 (50.0)	0.913	21 (52.5)	0.738
	>60	41	24 (58.5)		21 (51.2)		20 (48.8)	
Histologic type	WD	70	37 (52.9)	0.349	37 (52.9)	0.349	36 (51.4)	0.756
	MD/PD	11	4 (36.4)		4 (36.4)		5 (45.5)	
T classification	T1 + T2	67	32 (47.8)	0.379	32 (47.8)	0.379	32 (47.8)	0.379
	T3 + T4	14	9 (64.3)		9 (64.3)		9 (64.3)	
N classification	pN0	62	32 (51.6)	0.798	34 (54.8)	0.198	35 (56.5)	0.070
	pN1-3	19	9 (47.4)		7 (36.8)		6 (31.6)	
Stage	I + II	55	25 (45.5)	0.176	26 (47.3)	0.381	27 (49.1)	0.689
	III + IV	26	16 (61.5)		15 (57.7)		14 (53.8)	
Local recurrence	Negative	71	32 (45.1)	0.014*	34 (47.9)	0.312	35 (49.3)	0.737
	Positive	10	9 (90.0)		7 (70.0)		6 (60.0)	
Nodal recurrence	Negative	61	26 (42.6)	0.019*	25 (41.0)	0.004*	25 (41.0)	0.004*
	Positive	20	15 (75.0)		16 (80.0)		16 (80.0)	
Distant metastatic recurrence	Negative	78	38 (48.7)	0.241	40 (51.3)	0.616	39 (50.0)	1.000
	Positive	3	3 (100.0)		1 (33.3)		2 (66.7)	

WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated.
* Significance.