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IL17RB expression is associated with malignant cancer behaviors and poor prognosis in oral cancer

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Abstract

Objectives: Previously, we demonstrated that IL17RB plays an essential role in lung cancer progression. This study aimed to determine whether IL17RB correlates with oral cancer and promotes oral cancer progression.

Subjects and Methods: IL17RB expression in oral cancer tissues and normal tissues was determined by immunohistochemistry staining, while the association of IL17RB expression with the clinicopathological characteristics of oral squamous cell carcinoma (OSCC) patients was analyzed and its correlation with progression-free survival and response to radiotherapy and chemotherapy in OSCC patients was also explored. Western blotting was performed to investigate the expression of IL17RB in various OSCC cell lines; moreover, transwell assay was performed to evaluate the effect of IL17RB expression on cell migration ability.

Results: In this study, we found that IL17RB was expressed higher in OSCC tissues compared to normal oral mucosa tissues and its expression was positively correlated with tumor size, lymph node metastasis, advanced cancer stage, and poor prognosis.

Shyng-Shiou F. Yuan and Chang-Wei Su contributed equally to this article.

In vitro study showed that IL17RB expression in OSCC cell lines as determined by Western blotting, was positively correlated with their migration ability.

Conclusion: Clinical and in vitro studies suggest that IL17RB might serve as an independent risk factor and a therapeutic target for oral cancer.

KEYWORDS
IL17RB, oral cancer, patient survival, therapeutic target

1 | INTRODUCTION

Oral squamous cell carcinoma (OSCC) constitutes the majority of cancer incidences in the oral cavity and has been a major health issue worldwide (Ghantous & Elnaaj, 2017; Rivera, 2015). According to the GLOBOCAN 2020 data, there were 177,757 death cases for lip and oral cavity cancer (Hsieh et al., 2012; Sung et al., 2021), which also ranked as the fourth commonest cancer type in male Taiwanese. OSCC treatment remains a challenge owing to the frequent occurrence of treatment resistance and cancer metastasis (Bai et al., 2021; Vig et al., 2015), so it is important to identify useful prognostic factors and therapeutic targets for oral cancer.

Accumulating evidence has shown a strong association between chronic inflammation and tumorigenesis in various cancer types, including oral cancer (Coussens & Werb, 2002). The interleukin receptor family members are distinct from other single-transmembrane domain protein families, and, upon interleukin binding, they function in multiple cellular processes, including inflammation, immunity, and cancer (Li et al., 2006). Among these, the interleukin 17 (IL-17) family (IL17A to IL-17F) and its distinct cytokine receptor family (IL-17Rs, including IL-17RA to IL-17RE) are crucial for the immune responses in a normal host and the pathogenetic diseases in human (Kawaguchi et al., 2004; Kolls & Lindén, 2004; Witowski et al., 2004). High expression of IL-17 has been reported in several cancer types including breast cancer (Bartlett et al., 2019), prostate cancer (Sfanos et al., 2008), and lung cancer (Yang et al., 2018).

The interactions between IL-17 ligands and receptors are quite complicated. IL-17A and IL-17B are the ligands for IL-17RA and IL17RB, respectively (Shi et al., 2000), with IL-17A binding to IL-17RA/17RC having higher affinity than IL-17B, while IL-17E (also known as IL-25) has higher affinity to IL17RB than IL-17B (Wright et al., 2008; Yao et al., 1995). It has been reported that IL17RB plays an important role in breast cancer progression (Huang et al., 2017) and blocking IL17RB inhibits pancreatic cancer cell invasion (Wu et al., 2015). The role of IL17RB in oral cancer, however, remains unclear, so in this study, we investigated whether IL17RB is involved in oral cancer behaviors and prognosis by clinical and in vitro studies.

2 | MATERIALS AND METHODS

2.1 | Cell culture

Human oral squamous cancer cell lines Ca9-22, HSC-3, OECM-1, CAL-27, and SAS were obtained and authenticated (genotype and phenotype authentication) by the Bioresource Collection and Research Center, Taiwan (www.bcrc.firdi.org.tw). Ca9-22, HSC-3, CAL-27, and SAS were grown in DMEM/F12 (1:1) medium, whereas OECM-1 was cultured in RPMI1640 medium. All the culture media were purchased from Thermo Fisher Scientific and supplemented with 10% (vol/vol) fetal bovine serum (FBS) (Biological Industries), 1% penicillin/streptomycin/amphotericin B (Biological Industries), and 1% Glutamine. All the cells were grown and maintained at 37°C in a 5% CO_2 incubator (Thermo Fisher Scientific). Cells were regularly tested for mycoplasma contamination.

2.2 | Cell migration

Transwell systems (Corning) were used to study the migration of various oral cancer cells. Oral cancer cells were seeded in the upper chamber (20,000 cells/well) containing serum-free medium, whereas serum-containing medium was added to the lower chamber. These cells were incubated 48 h for cell migration assay, and at the end point, cells remaining in the upper chamber were removed using cotton swabs while cells migrating to the lower chamber were fixed and stained with crystal violet solution (4% paraformaldehyde, 1% methanol, 0.01% crystal violet) for 30 min before image capture using light microscopy. The percentage of the migrated cells was calculated by ImageJ software (http://imagej.net/ImageJ).

2.3 | Western blotting

Cells were harvested using trypsin and the cells were then lysed in ice-cold lysis buffer [50 mm Tris (pH 7.4), 120 mm NaCl, 1 mm EDTA, 0.5% NP40, and 50 mm NAF] plus protease inhibitors. The extracts were centrifuged at 12,000 *g* for 10 min at 4°C. Protein concentration was quantified using Pierce BCA protein assay kit

(23,225; Thermo Fisher Scientific); then, 20 μg blot samples of protein lysates were loaded and separated in SDS-PAGE and transferred onto nitrocellulose membrane, the blots then blocked with 2% skim milk in TBST and incubated with primary antibody rabbit anti-human IL17RB overnight (GTX127368; Genetex, dilution factor 1:1000), mouse anti-human beta-actin (A5441; Sigma-Aldrich, dilution factor 1:5000). After washing with TBST three times, the blots were incubated with secondary antibody goat anti-rabbit IgG (GTX213110-01; Genetex, dilution factor 1:5000) and anti-mouse IgG antibody (GTX213111-01; Genetex, dilution factor 1:5000). Finally, chemiluminescent signals were captured after washing with TBST three times.

2.4 | Patients

Between September 2002 and December 2011, a total of 107 OSCC patients (99 men and 8 women) from the Department of Oral and Maxillofacial Surgery of Kaohsiung Medical University Hospital (Kaohsiung, Taiwan) were enrolled in this study, with a median follow-up time of 40 months (range, 2.4–137.4 months). The non-cancerous tissues were normal oral epithelia from oral fibroma patients. The inclusion criteria for non-cancerous tissues were those without oral pre-cancer or cancer history. All the patients involved in the study were at M0 status. G*Power version 3.1.9.7 was used to estimate the power (Franz, Universitat Kiel) to consider the goodness of tests: contingency tables were found for IL17RB expression with clinicopathological characteristics with α of 0.05, and the estimated effect size w of 0.46. In this study, we recruited 107 OSCC patients to achieve sufficient power of at least 90%.

The current study was approved by the Institutional Review Board of Kaohsiung Medical University Hospital (approval no. KMUH-IRB-20130300) and patient informed consent was waived by the Institutional Review Board due to the retrospective nature of the study. OSCC pathology was determined by two pathologists independently, and the final diagnoses were made using clinical and histological data. Patients without previous history of any treatment for oral cancer were included. Patients who were <18 or >80 years of age were excluded. Baseline characteristic data included patient age, sex, tumor location, grade, tumor size, lymph node metastases, and tumor stage; additionally, substance use such as alcohol consumption, betel quid chewing or cigarette smoking, and adjunct treatment details were recorded (Table 1). The mean age of the study group was 51.4 years and median age was 51 years (age range, 31–76 years). Clinical staging of the patients was determined using the TNM staging system according to the 1992 criteria of the American Joint Committee on Cancer/Union for International Cancer Control, with primary tumor locations being buccal mucosa (77.6%) and tongue (22.4%). All of the patients received surgery as primary treatment, and some patients received adjuvant treatment such as radiotherapy and chemotherapy. A total of 29 patients received adjuvant chemotherapy, with the chemotherapy regimen consisting of cisplatin or carboplatin with or without the addition of 5-fluorouracil or paclitaxel or docetaxel. A total of 47 patients received adjuvant intensity-modulated radiotherapy, and the scheduled doses were given once per day, 5 days per week. Postoperative patients received the planned course of adjuvant radiotherapy of 60–66 Gy in 2-Gy fractions to the post-operation high-risk region.

2.5 | IHC staining

IHC staining for IL17RB was performed on a Bond-Max autostainer (Leica Microsystems) as described previously (Hung et al., 2016; Wang et al., 2021). Briefly, resections were incubated with IL17RB antibody which was a gift from Professor Wen-Hwa Lee (Lee et al., 2022), and color development was performed with 3,3′-diaminobenzidine tetrahydrochloride. Slides were counterstained with hematoxylin, and images were captured using a Nikon Eclipse Ti microscope (Nikon). Negative control was performed in the same process but without staining with MRE11 antibody. The percentage of positively stained tumor cells was analyzed semi-quantitatively by evaluating tumor sections. The total proportion of positively stained cells were sub-classified into four categories of 0 to 4: 0 (0–4%; when 0% to 4% cells stained positive), 1 (5%–24%; when 5% to 24% cell stained positive), 2 (25%–49%; when 25%–49% cell stained positive), 3 (50%–74%; when 50% to 74% cells stained positive), or 4 (75%–100%; when >75% cells stained positive). In addition, intensity of immunostaining was determined as 0 (negative), 1 (weak), 2 (moderate), or 3 (strong). The total score was designated as the percentage of positively stained cells multiplied by the weighted intensity of staining for each sample. For further statistical analysis, scores ≤9 were categorized as low IL17RB expression, and >9 were categorized as high IL17RB expression (cut point at median).

2.6 | The Cancer Genome Atlas (TCGA) database analysis

IL17RB RNA-Seq datasets of the head and neck squamous cell carcinoma (HNSC) in TCGA database were retrieved from TCGA website (Project ID: TCGA-HNSC; http://portal.gdc.cancer.gov/). Kaplan–Meier curves were applied to compare the progression-free survival probability between low expression and high expression of IL17RB cohorts from the TCGA-HNSC patients.

2.7 | Statistical analysis

In this study, the estimated odds ratio (OR) was used to summarize the relationship between IL17RB expression and clinicopathological features of patients. Hazard ratios (HR) and 95% confidence intervals (CI) from univariate and multivariable analyses using the Cox proportional hazard regression models were performed to calculate the associations between progression-free survival and clinical

TABLE 1 Association of IL17RB expression with clinicopathological characteristics of OSCC patients using logistic regression.

		IL17RB				
		Low	High			
Variables	Categories	*N* (%)	*N* (%)	*p*-Value	Crude OR (95% CI)	Adj OR (95% CI)
Histopathological Grade	I	21 (28.0)	54 (72.0)	0.13	1	—
	II	5 (55.6)	4 (44.4)		0.31 (0.08–1.27)	
	Missing = 23					
Tumor size	T1–T2	28 (39.4)	43 (60.6)	0.03	1	1
	T3–T4	6 (16.7)	30 (83.3)		3.26 (1.20–8.83)	2.55 (0.91–7.15)
Lymph node metastasis	No	31 (38.3)	50 (61.7)	0.01	1	1
	Yes	3 (11.5)	23 (88.5)		4.75 (1.32–17.16)	3.74 (1.01–13.95)
Pathologic stage	I + II	25 (43.1)	33 (56.9)	0.007	1	—
	III + IV	9 (18.4)	40 (81.6)		3.37 (1.38–8.20)	
Radiotherapy	No	21 (36.8)	36 (63.2)	0.29	1	—
	Yes	12 (25.5)	35 (74.5)		1.70 (0.73–3.97)	
	Missing = 3					
Chemotherapy	No	23 (30.7)	52 (69.3)	0.81	1	—
	Yes	10 (34.5)	19 (65.5)		0.84 (0.34–2.09)	
	Missing = 3					
Sex	Female	3 (37.5)	5 (62.5)	0.71	1	—
	Male	31 (31.3)	68 (68.7)		1.32 (0.30–5.86)	
Alcohol drinking	No	11 (35.5)	20 (64.5)	0.65	1	—
	Yes	23 (30.4)	53 (69.7)		1.27 (0.52–3.07)	
Betel quid chewing	No	8 (38.1)	13 (61.9)	0.60	1	—
	Yes	26 (30.2)	60 (69.8)		1.42 (0.53–3.84)	
Cigarette smoking	No	3 (21.4)	11 (78.6)	0.54	1	—
	Yes	31 (33.3)	62 (66.7)		1.83 (0.48–7.05)	

Note: Chi-square was used in this analysis. Adjusted OR were calculated, adjusting tumor size and lymph node metastasis from multivariate analysis.

characteristics. In the multivariate analysis, the qualifying criteria for inclusion variables were those with *p*-values of less than 0.05 in the univariate analysis. Experimental data obtained were analyzed using the SPSS 20.0 statistical package for PC (SPSS) and GraphPad Prism v5.0 (GraphPad Software Inc.). All *p*-values less than 0.05 were considered statistically significant. $^{*}p<0.05$, $^{**}p<0.005$, $^{***}p<0.001$, $^{****}p<0.0001$.

3 | RESULTS

3.1 | The expression of IL17RB in OSCC patients

To evaluate the expression of IL17RB protein in oral cancer tissues, immunohistochemical analysis was performed. Our data showed that IL17RB expression was lower in normal oral mucosa tissues compared with its expression in oral cancer tissues (Figure 1a,b) ($p=0.0002$). Using TCGA-HNSC dataset, we observed a higher IL17RB mRNA expression in head and neck squamous carcinomas compared to normal samples (Figure 1c). Survival analyses according to IL17RB protein expression in oral cancer tissues showed that the high IL17RB expression group had decreased progression-free survival with *p*-value of 0.0001 (Figure 1d).

3.2 | High IL17RB expression in oral cancer tissue was associated with radioresistance and chemoresistance

We further analyzed the correlation between IL17RB expression and various clinical behaviors. As shown in Table 1, high IL17RB expression in oral cancer tissues was associated with larger tumor size, increased lymph node metastasis ($p=0.01$), and advanced cancer stage. Patients with high IL17RB expression had a higher possibility of receiving radiotherapy and chemotherapy, as well as using alcohol, chewing betel quid, and smoking cigarettes (Table 1). Moreover, larger tumor size (HR = 2.18, 95% CI = 1.38–3.42, $p=0.0007$), lymph node metastasis (HR = 1.56, 95% CI = 0.95–2.57, $p=0.08$), and high IL17RB expression (HR = 2.96, 95% CI = 1.85–4.4, $p<0.0001$) in cancer tissues were

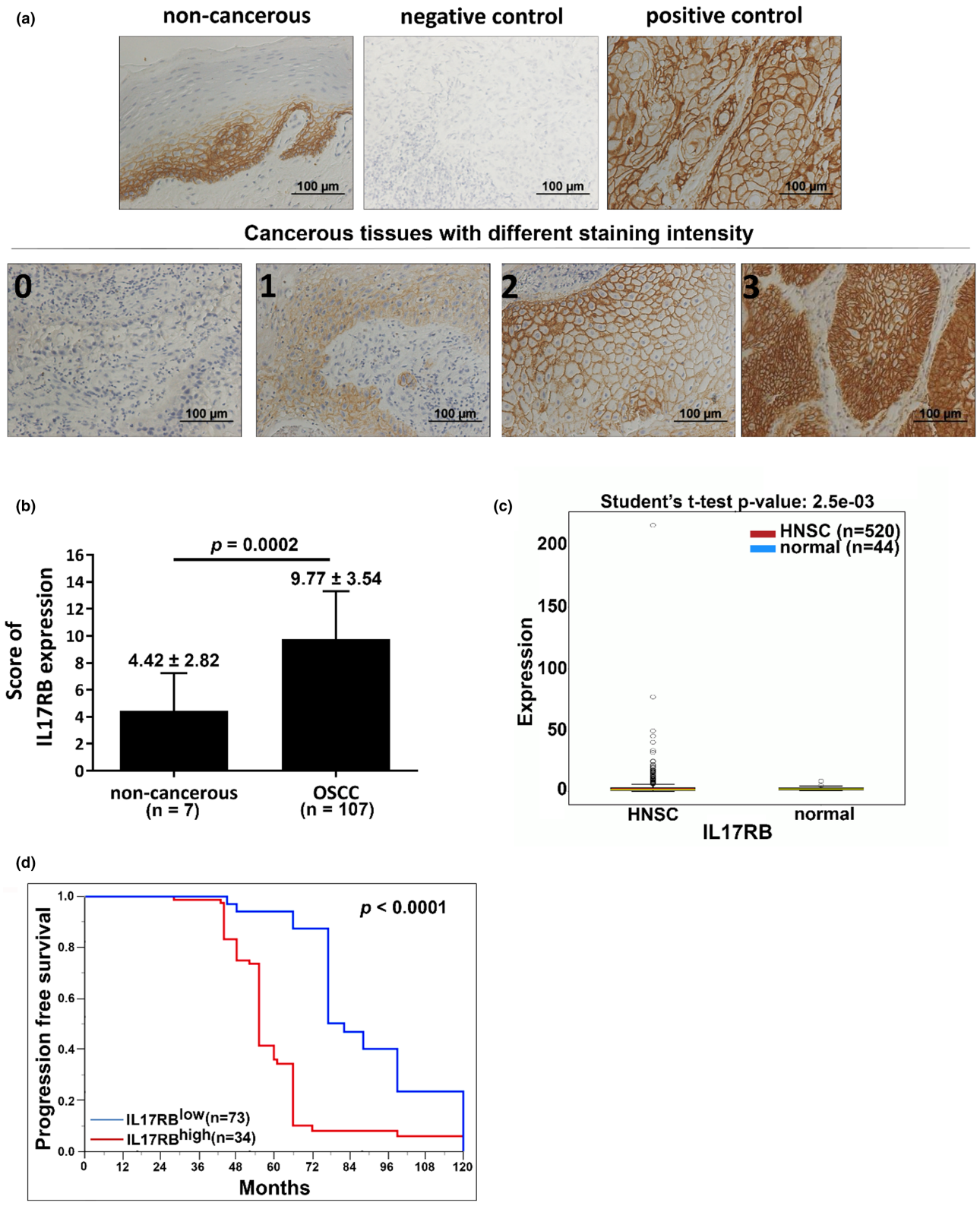

FIGURE 1 IL17RB expression was elevated in oral cancer tissues and associated with decreased progression-free survival. (a) Immunohistochemical (IHC) staining for IL17RB in non-cancerous and cancerous tissues. Slides were stained using methods described in Materials and methods. Staining was scored according to the staining intensity in four quantitative categories (0 = absent staining; 1 = weak staining; 2 = moderate staining; 3 = strong staining). The expression of MRE11 in normal and negative controls are also shown. The representative photograph is shown with 400× magnification. (b) Quantitative result for IL17RB expression in oral non-cancerous and cancer tissues. (c) Increased IL17RB mRNA expression in oral cancer tissues, in comparison with normal tissues, reported in online databases. (d) Progression-free survival for high and low IL17RB protein expression groups from our clinical study. Survival curves were generated using the Kaplan–Meier method and the p-values were calculated using the log-rank test.

TABLE 2 Univariate and multivariable analyses of progression-free survival for OSCC patients.

			Univariate		Multivariable	
Variable	Categories	*N*	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
Histopathological Grade	I	75	1		–	–
	II	9	0.98 (0.47–2.01)	0.97	–	–
	Missing = 23					
Tumor size	T1–T2	71	1	0.0007	1	0.0019
	T3–T4	36	2.18 (1.38–3.42)		2.08 (1.31–3.31)	
Lymph node metastasis	No	81	1	0.08	–	–
	Yes	26	1.56 (0.95–2.57)		–	–
IL17RB	Low	34	1	<0.0001	1	<0.0001
	High	73	2.96 (1.85–4.74)		2.93 (1.81–4.72)	
Radiotherapy	No	57	1			
	Yes	47	0.95 (0.63–1.43)	0.80		
Chemotherapy	No	75	1			
	Yes	29	1.33 (0.83–2.14)	0.24		
Sex	No	8	1			
	Yes	99	0.89 (0.43–1.84)	0.75		
Alcohol drinking	No	31	1			
	Yes	76	1.06 (0.68–1.66)	0.80		
Betel quid chewing	No	22	1			
	Yes	85	1.07 (0.67–1.73)	0.77		
Cigarette smoking	No	14	1			
	Yes	93	0.75 (0.42–1.33)	0.32		

Note: Adjust HR were calculated, adjusting tumor size and IL17RB from multivariate analysis.

the risk factors for decreased progression-free survival in oral cancer patients, as determined by univariate cox regression analysis (Table 2); however, in multivariable analysis, only larger tumor size (HR = 2.08, 95% CI = 1.31–3.31, $p = 0.0019$), and high IL17RB expression (HR = 2.93, 95% CI = 1.81–4.72, $p < 0.0001$) in cancer tissues were significant risk factors (Table 2).

To understand whether IL17RB is an independent risk factor for progression-free survival of cancer patients, both (1) the association between clinicopathological characteristics of OSCC patients with or without adjuvant therapies and (2) the associations between IL17RB expression and progression-free survival in patients with or without receiving adjuvant therapies were analyzed. First, the association between clinicopathological characteristics of OSCC patients with chemotherapy and progression-free survival, univariate and multivariable analyses were performed as shown in Table 3. In patients who received chemotherapy, univariate analysis showed that high IL17RB expression was significantly associated with progression-free survival (HR = 18.29, 95% CI = 2.41–138.82, $p = 0.0049$). For the patients who received radiotherapy, factors significantly associated with progression-free survival included larger tumor size (HR = 2.43, 95% CI = 1.22–4.77, $p = 0.0110$) and high expression of IL17RB (HR = 2.57, 95% CI = 1.23–5.35, $p = 0.0119$) (Table 4). We also analyzed IL17RB expression on the survival of patients receiving chemotherapy or radiotherapy. As shown in Figure 2, patients with high IL17RB expression had decreased progression-free survival with (Figure 2a, $p < 0.0001$) or without (Figure 2b, $p < 0.0001$) chemotherapy. Similarly, patients with high IL17RB expression had decreased progression-free survival with (Figure 3a, $p = 0.0023$) or without (Figure 3b, $p < 0.0001$) radiotherapy. These results suggest that IL17RB is an independent prognostic factor for oral cancer.

3.3 | Correlation between high IL17RB expression and cell migration in various oral cancer cell lines

The expression of IL17RB in various oral cancer cell lines was determined by western blotting. High IL17RB expression levels were observed in OECM-1 and Ca9-22 cell lines, while HSC-3, CAL-27, SAS cell lines expressed lower levels of IL17RB (Figure 4a). As shown in Figure 4b, cell migration ability of higher IL17RB-expressing OECM-1 and Ca9-22 cells was significantly higher than that of lower IL17RB-expressing SAS, HSC-3, and CAL-27 cell lines. Taken together, our data suggest that IL17RB could play a role in regulating cell migration in oral cancer cells.

TABLE 3 Association between clinicopathological characteristics of OSCC patients with and without chemotherapy and progression-free survival.

			Univariate		Multivariable	
Variable	**Categories**	***N***	**HR (95% CI)**	***p*-Value**	**HR (95% CI)**	***p*-Value**
Chemotherapy (+)						
Tumor size	T1–T2	12	1	0.061	–	–
	T3–T4	17	2.47 (0.96–6.36)		–	–
Lymph node metastasis	No	19	1	0.34	–	–
	Yes	10	1.52 (0.65–3.58)		–	–
IL17RB	Low	10	1	0.0049	–	–
	High	19	18.29 (2.41–138.82)		–	–
Radiotherapy	No	14	1	0.97	–	–
	Yes	15	1.02 (0.43–2.40)		–	–
Sex	Female	4	1	0.30	–	–
	Male	25	0.58 (0.17–1.73)		–	–
Alcohol drinking	No	17	1	0.74	–	–
	Yes	12	0.85 (0.34–2.16)		–	–
Betel quid chewing	No	2	1	0.55	–	–
	Yes	27	0.64 (0.15–2.80)		–	–
Cigarette smoking	No	6	1	0.53	–	–
	Yes	23	0.73 (0.28–1.94)		–	–
Chemotherapy (–)						
Tumor size	T1–T2	57	1	0.0073	1	0.0091
	T3–T4	18	2.18 (1.23–3.87)		2.17 (1.21–3.87)	
Lymph node metastasis	No	61	1	0.13	–	–
	Yes	14	1.63 (0.86–3.11)		–	–
IL17RB	Low	23	1	0.0011	1	0.0012
	High	52	2.44 (1.43–4.15)		2.44 (1.42–4.19)	
Radiotherapy	No	43	1	0.57	–	–
	Yes	32	0.87 (0.54–1.40)		–	–
Sex	Female	4	1	0.66	–	–
	Male	71	1.25 (0.45–3.47)		–	–
Alcohol drinking	No	19	1	0.54	–	–
	Yes	56	1.18 (0.69–2.00)		–	–
Betel quid chewing	No	20	1	0.72	–	–
	Yes	55	1.10 (0.65–1.85)		–	–
Cigarette smoking	No	8	1	0.60	–	–
	Yes	67	0.82 (0.39–1.73)		–	–

Note: Adjust HR were calculated, adjusting tumor size and IL17RB from multivariate analysis.

4 | DISCUSSION

The etiology of oral cancer is multifactorial (Tagliabue et al., 2021). In this study, the role of IL17RB in oral cancer patients was assessed and high IL17RB expression in cancer tissues was associated with shorter progression-free survival, although with no sex preference (Table 1). Its expression was also not associated with risk factors for oral cancer including alcohol drinking (Ogden, 2005), betel quid chewing (Zhang & Reichart, 2007), or cigarette smoking (Ko et al., 1995) that have all been reported to be involved in oral cancer.

The potential application of IL17RB has been implicated in several malignancies. IL17RB expression was upregulated in invasive ductal carcinoma of breast (Furuta et al., 2011) and associated with decreased survival rate in breast cancer patients (Laprevotte et al., 2017) (Huang et al., 2014). Furthermore, IL17RB expression was associated with HER2 amplification and survival rate in the patients with high expression of IL17RB and HER2 was the lowest

TABLE 4 Association between clinicopathological characteristics of OSCC patients with and without radiotherapy and progression-free survival.

			Univariate		Multivariable	
Variable	**Categories**	***N***	**HR (95% CI)**	***p*-Value**	**HR (95% CI)**	***p*-Value**
Radiotherapy (+)						
Tumor size	T1–T2	31	1	0.011	1	0.0013
	T3–T4	16	2.42 (1.22–4.77)		3.38 (1.61–7.11)	—
Lymph node metastasis	No	36	1	0.44	—	—
	Yes	11	1.37 (0.61–3.08)		—	—
IL17RB	Low	12	1	0.0119	1	0.0019
	High	35	2.57 (1.23–5.35)		3.41 (1.57–7.40)	—
Chemotherapy	No	32	1	0.28	—	—
	Yes	15	1.43 (0.74–2.73)		—	—
Sex	Female	0	—	—	—	—
	Male	47	—		—	—
Alcohol drinking	No	38	1	0.36	—	—
	Yes	9	0.70 (0.32–1.51)		—	—
Betel quid chewing	No	11	1	0.35	—	—
	Yes	36	1.45 (0.66–3.16)		—	—
Cigarette smoking	No	3	1	1.00	—	—
	Yes	44	1.00 (0.31–3.27)		—	—
Radiotherapy (–)						
Tumor size	T1–T2	38	1	0.0205	1	0.18
	T3–T4	19	2.08 (1.12–3.86)		1.55 (0.81–2.95)	
Lymph node metastasis	No	44	1	0.058	—	—
	Yes	13	1.88 (0.98–3.60)		—	—
IL17RB	Low	21	1	<0.0001	1	0.0003
	High	36	3.60 (1.91–6.78)		3.35 (1.74–6.45)	
Chemotherapy	No	43	1	0.63	—	—
	Yes	14	1.20 (0.58–2.45)		—	—
Sex	Female	8	1	0.93	—	—
	Male	49	0.97 (0.45–2.07)		—	—
Alcohol drinking	No	22	1	0.21	—	—
	Yes	35	1.45 (0.81–2.58)		—	—
Betel quid chewing	No	11	1	0.41	—	—
	Yes	46	1.33 (0.68–2.59)		—	—
Cigarette smoking	No	11	1	0.36	—	—
	Yes	46	0.72 (0.36–1.44)		—	—

Note: Adjust HR were calculated, adjusting tumor size and IL17RB from multivariate analysis.

(Huang et al., 2014). In pancreatic cancer, a low IL17RB expression group, compared with a high IL17RB-expression group, was associated with better overall survival after receiving chemotherapy (Song et al., 2019). In this present study, we also found that high IL17RB expression had decreased progression-free survival in oral cancer patients (Figure 1d). However, our study showed that high IL17RB expression led to decreased progression-free survival in oral cancer patients no matter receiving chemotherapy or not (Figure 2). Furthermore, we also found that high IL17RB level resulted in poor survival in oral cancer patients with or without receiving radiotherapy (Figure 3).

Other than promoting cancer cell proliferation and survival, IL-17B/IL17RB signaling pathway induces stemness and epithelial-to-mesenchymal transition in gastric cancer cells via the AKT/GSK-3β/ β-catenin pathway and upregulation of SOX2 and OCT4 proteins (Bie et al., 2016). Indeed, our previous study also demonstrated that IL17RB-mediated activation of ERK/GSK-3β/ β-catenin pathway is important to maintain the two key transcription

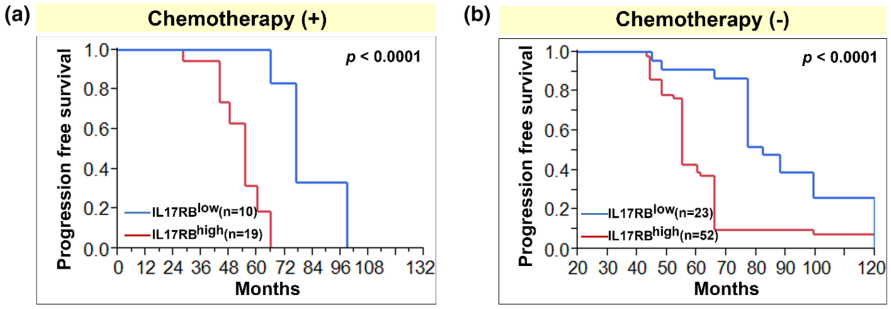

FIGURE 2 Effect of IL17RB expression on progression-free survival of oral cancer patients with chemotherapy (a) and without chemotherapy (b).

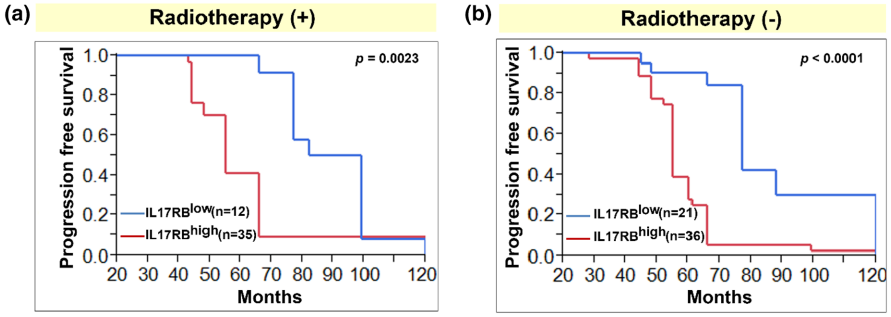

FIGURE 3 Effect of IL17RB expression on progression-free survival of oral cancer patients with radiotherapy (a) and without radiotherapy (b).

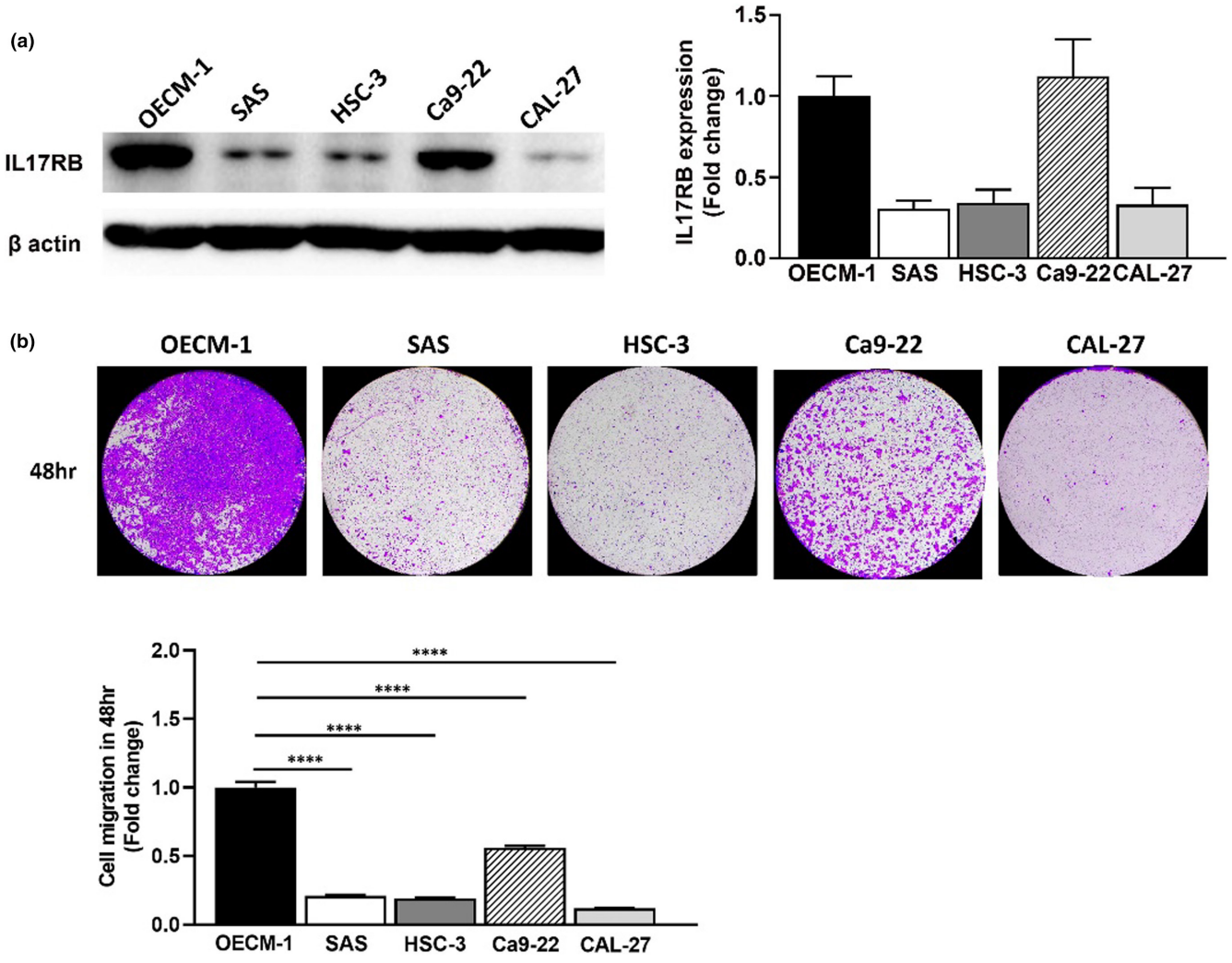

FIGURE 4 Expression levels of IL17RB in oral cancer cell lines were positively associated with cell migration ability. (a) IL17RB showed highest expression in OECM-1 cells. (b) The relationship between IL17RB expression and cell migration ability in oral cancer cells was analyzed after 48 hr incubation in transwell. Statistical difference was determined by Student's *t* test. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

factors, Snail and Twist, for EMT induction and in vivo metastasis in lung cancer cells (Yang et al., 2018). These in vitro results were also confirmed in a cohort of 139 primary lung tumor samples that IL17RB expression was positively correlated with Snail and Twist expression (Yang et al., 2018). IL17RB and its ligands IL17B promote tumorigenesis in breast cancer cells by activating NF-κB through TRAF6 and blocking this pathway with either IL17RB or IL17B antibodies resulted in the reduction of breast cancer tumorigenicity (Huang et al., 2014). Similarly, overexpression of IL17RB and its ligand IL17B in pancreatic cancer cells activates the ERK signaling pathways followed by activation of chemokine CC ligand 20, chemokine CXC ligand 1, trefoil factor 1 and IL-8, and thus activates pancreatic cancer cell metastasis and invasion (Wu et al., 2015). These chemokines might be secreted by tumor-infiltrating cells as a result of IL17RB stimulation and contribute to macrophages and endothelial cells recruitment to stimulate cancer progression.

This present study has some limitations, including data regarding socioeconomic factors not being collected and analyzed while data were collected only from a single cancer center, thus requiring more extensive multi-center studies to reinforce the present findings. Also, various prognostic factors, other than IL17RB, have been reported to be associated with prognosis of cancer patients. In oral cancer, margins are invaded more often than head and neck cancer in any other sites (Binahmed et al., 2007; Sutton et al., 2003), and the invaded surgical margins may have significant impact on patient prognosis (Kansy et al., 2017; Lang et al., 2022; McMahon et al., 2011). Comorbidities status is also a critical factor for patient prognosis. In a study with a total of 16,676 oral cavity cancer patients, 21.3% of the patients had certain types of comorbidity during the year before cancer diagnosis, including diabetes mellitus (10%), peptic ulcer disease (4.5%), and liver disease (3.8%). Severe comorbidities significantly increased the mortality in oral cancer patients regardless of the stages (Jariod-Ferrer et al., 2019; Yang & Warnakulasuriya, 2016). While surgical margin and comorbidity issues were not included in our study, they may have a significant impact on patient survival and worthy of further comprehensive studies.

One other limitation of this study is that AJCC 7th edition was adopted for TNM staging. In 2017, the American Joint Committee on Cancer staging system had introduced 8th edition with several major changes. Accordingly, primary tumors formerly categorized as T1 are upgraded to T2 in the presence of depth of invasion (DOI) >5 mm beyond the basement membrane, and primary tumors formerly categorized as T2 are upgraded to T3 when DOI >10 mm. Recent studies showed that around 25% of the patients were upgraded and 83% of them had higher risk scores, when followed AJCC 8th edition (Amit et al., 2019; Rahman et al., 2021). Overall risk score and TNM stage following 8th edition have more significant correlation with survival rate in comparison with the previous TNM stage edition (Amit et al., 2019).

5 | CONCLUSION

This is the very first study to explore the role of IL17RB in oral cancer. While our study suggests that IL17RB is a poor prognostic biomarker for oral cancer, the molecular mechanism should be explored by further in vitro and in vivo studies; furthermore, larger-scale prospective studies to validate the possible role of IL17RB as a prognostic factor, as well as a therapeutic target for oral cancer patients, are required. Also, the stratified analysis for confirmation that IL17RB is an independent prognostic factor for oral cancer must be interpreted in the context of study limitations, namely small sample size and limited statistical power. Studies thus far have been underpowered to detect this effect, and primary studies with much larger sample sizes will be required to properly investigate this association.

AUTHOR CONTRIBUTIONS

Yen-Yun Wang: Conceptualization; data curation; formal analysis; visualization; writing – original draft; writing – review and editing; project administration; supervision; investigation; methodology; software; validation; funding acquisition; resources. **Shyng-Shiou F. Yuan:** Methodology; conceptualization; resources; supervision; software; investigation; validation; data curation; formal analysis; visualization; writing – original draft; writing – review and editing; project administration; funding acquisition. **Chang-Wei Su:** Methodology; resources; investigation; validation; supervision; writing – review and editing. **Leong-Perng Chan:** Methodology; investigation; resources; writing – review and editing. **Hieu D. H. Nguyen:** Validation; investigation; data curation; methodology; writing – review and editing. **Yuk-Kwan Chen:** Investigation; writing – review and editing; supervision. **Je-Kang Du:** Investigation; supervision; writing – review and editing. **Kuang-Hung Cheng:** Investigation; writing – review and editing; supervision.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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