Biological information and functional analysis reveal the role of discoidin domain receptor 1 in oral squamous cell carcinoma

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Objectives. This study aimed to establish a framework for the role of discoidin domain receptor 1 (DDR1) in oral squamous cell carcinoma (OSCC) through biological data and functional analysis.

Study Design. The GSE31056 series of the Gene Expression Omnibus database and UALCAN website were used to assess DDR1 expression in head and neck squamous cell carcinoma (HNSCC) and OSCC. DDR1 RNA sequencing data for 260 HNSCC samples from The Cancer Genome Atlas were overlaid to evaluate its association with tumor progression and prognosis. To identify the function of DDR1 in OSCC, 38 patients with OSCC were followed for 8 years and immunohistochemical analysis, western blotting, Cell Counting Kit-8, and colony formation assays were conducted on OSCC cell lines to reveal DDR1 expression and function.

Results. DDR1 was overexpressed in HNSCC and OSCC tumor specimens and its expression correlated with overall survival and T-stage classification ($P = .049$, $P = .0316$). Furthermore, DDR1 was related to OSCC tumor growth because its expression increased with the T-stage level ($P = .0071$) but not N-stage level, histologic stage, or recurrence ($P > .05$). DDR1 was highly expressed in OSCC cell lines and promoted cell proliferation, which was repressed by nilotinib ($P < .05$).

Conclusions. DDR1 has an oncogenic role in OSCC and might be a novel target for anti-OSCC therapy. (Oral Surg Oral Med Oral Pathol Oral Radiol 2021;131:221−230)

With an estimated 354,864 new cases and 177,384 deaths reported annually, head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide, with oral squamous cell carcinoma (OSCC) being among the most frequent types of HNSCC, excluding nonmelanoma skin cancer.[1] Major risk factors include tobacco and alcohol consumption and human papillomavirus infection. In Southeast Asia, chewing betel quid is also a crucial risk factor, because 75% of approximately 59,000 males annually affected by oral cancer have a history of combined smoking-drinking-betel quid exposure.[2−5] Major treatment options for OSCC include various combinations and sequences of surgery, radiotherapy, chemotherapy, and the use of targeted therapeutic agents.[6] However, the 5-year survival rate for oral cavity cancer is only approximately 50%, depending on the stage at diagnosis.[2,7] Over the past several decades, a number of specific inhibitors of receptor tyrosine kinases (RTKs) have been developed and approved by the US Food and Drug Administration as targeted therapies for certain cancers.[8]

Discoidin domain receptor 1 (DDR1) is a transmembrane receptor that belongs to the RTK family and consists of an extracellular discoidin homology domain, a discoidin-like domain, an extracellular juxtamembrane region, and a catalytic tyrosine kinase domain.[9] DDRs are the only RTKs specifically bound and activated by collagens and can thereby delay or sustain receptor phosphorylation to mediate downstream molecular signaling pathways.[10] DDR1 can be activated by fibrillar collagen (types I−III and V) and also by nonfibrillar type IV collagen.[9] DDR1 was first discovered in *Dictyostelium discoideum* by Breuer and Siu in 1981.[11] Subsequent studies determined that DDR1 is mainly expressed in epithelial cells and is indispensable for embryonic development.[12] Numerous studies have also indicated that DDR1 is involved in many disease processes, such as fibrotic diseases, osteoarthritis, and atherosclerosis.[13] Over the past few years, DDR1 has been reported in multiple tumor types, and growing evidence has shown that DDR1 plays an important role in cancer pathogenesis, including tumor proliferation, invasion, migration, and metastasis. For instance, in pancreatic ductal adenocarcinoma, inhibition of DDR1 is reported to reduce collagen-mediated tumorigenicity[14]; in thyroid cancer, DDR1 can regulate cell differentiation[15]; and in hepatocellular carcinoma, DDR1 has been shown to play a role in invasion.[16] These previous findings clearly demonstrate that DDR1 plays a vital role

Statement of Clinical Relevance

This study aimed to identify the role of discoidin domain receptor 1 (DDR1), an oncogenic factor, in oral squamous cell carcinoma through bioinformatics and functional analysis and to determine whether it can serve as a novel target for anti−oral squamous cell carcinoma therapy.

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in tumor development. In addition, recent studies have shown that DDR1-targeted therapy may be feasible in the near future as part of antineoplastic protocols.[17] Specifically, tyrosine kinase inhibitors (TKIs) such as nilotinib, have been approved for the treatment of chronic myelogenous leukemia,[18] and apatinib was effective in the maintenance therapy of extensive-stage small cell lung cancer.[19] However, at the same time, the specific mechanism of DDR1 in cancer prognosis remains unclear.[20] For instance, the number of relevant studies regarding DDR1 in OSCC are limited. Thus, our goal was to determine the role of DDR1 in OSCC using functional and biological information analyses.

MATERIALS AND METHODS

OSCC clinical specimens

A total of 34 sets of OSCC primary tumor specimens and their corresponding metastatic lymph nodes and normal samples were collected in accordance with the guidelines of the Institutional Review Board from the Department of Oral and Maxillofacial Surgery of the Second Xiangya Hospital, Central South University, Changsha, Hunan, China. The 34 tumor specimens were classified according to the International Union Against Cancer tumor-node-metastasis classification system before treatment.[21] Criteria for study inclusion were as follows: (1) patients who were diagnosed pathologically with OSCC, including labial mucosa cancer, buccal mucosa cancer, oral floor cancer, alveolar ridge and gingiva cancer, tongue cancer, and hard palate cancer; (2) patients who were diagnosed for the first time between 2011 and 2013; (3) patients whose complete data were retained after surgery and who were followed up until December 31, 2018; (4) patients who never received any radiotherapy, chemotherapy, or biological therapy before surgery. Patients (1) with severe systemic diseases such as cardiac disease, diabetes, and hypertension and (2) who were diagnosed with other types of HNSCC such as oropharyngeal cancer were excluded from this study. The OSCC specimens included 22 tongue cancers, 3 oral floor cancers, 3 gingiva cancers, and 6 buccal mucosa cancers, which were pathologically diagnosed after surgery. The metastatic lymph node tissues and paracancerous tissues (>1.5 cm away from the primary site) were collected from the same patients and were pathologically diagnosed as metastatic lymph node tissues of oral cancer and normal mucosal tissues free of cancer.

Histologic and immunohistochemical analysis

All tissues, including the primary tumors, metastatic lymph nodes, and normal samples, were fixed, paraffin-embedded, and sectioned (4 μm). The tissue sections were then deparaffinized, rehydrated, and incubated with Tris-EDTA buffer (pH 9.0) for antigen retrieval. The sections were blocked with 3% hydrogen peroxide and then incubated with primary DDR1 antibody (5538s; Cell Signaling Technology, Boston, MA) overnight at 4°C. Following incubation with the appropriate horseradish peroxidase−conjugated secondary antibodies, 0.03% 3,3′-diaminobenzidine was used as a substrate to visualize the immunostaining. All sections were counterstained with hematoxylin and photomicrographic images were captured for subsequent analysis. Sections incubated with only phosphate-buffered saline (PBS) were used as negative controls, and breast cancer tissues diagnosed pathologically were used as positive controls.

Two experienced pathologists independently assessed the immunohistochemical staining, and a final score was obtained based on their findings. The immunoreactivity scoring system considered (1) the number of positive-stained cells (0 = ≤5%, 1 = 6%-25%, 2 = 26%-50%, 3 = 51%-75%, and 4 = ≥76%) and (2) the intensity of staining (0 = no staining, 1 = weak staining, 2 = modeate staining, and 3 = strong staining). The values of (1) and (2) were multiplied to obtain the final immunoreactive scores.[22] The median immunohistochemical score was used as the criteria of high/medium/low distribution for survival analysis.

Cell culture

Oral cancer cell lines of SCC-4 and CAL-27 (ATCC) were gifts from the Center for Molecular Medicine, Xiangya Hospital, Central South University, China, and were cultured in Dulbecco's Modified Eagle Medium with 10% fetal bovine serum and 1% penicillin-streptomycin in a 5% CO_2 37°C cell culture incubator and routinely evaluated for mycoplasma contamination.

Western blotting

The SCC-4 and CAL-27 cells were exposed to nilotinib-PBS (1, 2, and 4 μg/μL) or PBS alone 24 h before total protein lysates were obtained using lysis buffer composed of RIPA buffer, protease inhibitor cocktail, and phosphatase inhibitor cocktail (Bimake, Houston, TX). The supernatant of the lysates was withdrawn after centrifugation and protein concentrations were detected using the bicinchoninic acid assay. The proteins were separated by sodium dodecyl sulfate−polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride membranes. The membranes were incubated overnight at 4°C with Tubulin (sc-23948, Santa Cruz, TX; 1:10,000) and DDR1 (CST, 5583T, 1:2000). Horseradish peroxidase−conjugated secondary antibodies were then used to visualize the protein bands.

Cell Counting Kit-8 assay

The SCC-4 and CAL-27 cells were exposed to nilotinib-PBS (0, 1, 2, 4 μg/μL). Cell proliferation was then detected using Cell Counting Kit-8 (CCK8, Selleck,

US) according to the manufacturer's instructions. Briefly, 4000 cells per well were seeded into 96-well plates with complete culture medium. After 48, 72, 96, and 120 h, 10 μL of the CCK8 reagent was added into the testing well and incubated for 2 h. The absorbance was measured at a wavelength of 450 nm.

Colony formation assay

For the colony formation assay, SCC-4 and CAL-27 cells (5000 cells/well) were seeded into 6-well plates, exposed to nilotinib-PBS (0, 1, 2, and 4 μg/μL), and cultured for 7 days at 37˚C in a culture hood. Colonies were fixed, stained with 1% crystal violet, and counted under a microscope.

Gene Expression Omnibus data acquisition and processing

The Gene Expression Omnibus database of the National Center of Biotechnology Information (https://www.ncbi.nlm.nih.gov/geo/) is a public genomics repository that contains high-throughput sequencing chip and microarray data.[23,24] After logging into the website and editing the search parameters, the GSE31056 series related to DDR1 expression in the OSCC profile was acquired.[25] The GSE31056 series included microarray data regarding 22 tumor tissue samples, 51 margin samples, and 23 normal tissue samples. The data information was downloaded, analyzed, and visualized using GraphPad Prism7 (GraphPad Software, San Diego, CA).

The Cancer Genome Atlas data processing and analysis

The Cancer Genome Atlas program (https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga) is a publicly available cancer genomics program that contains genetic data on over 20,000 primary cancer specimens and their matched normal samples, spanning 33 different types of cancer. The TCGA HNSCC data, including RNA sequencing (RNA-Seq) expression, gene mutation, and matched clinical data, were obtained and processed using the RTCGAToolbox package (20160128) available in R (https://cran.r-project.org/) Lucent Technologies, the US as previously described.[26] For DDR1 differential expression analysis, patients were classified as DDR1-high or DDR1-low based on their median DDR1 expression scores. For tumor staging analysis, only samples with known tumor grade information were retained. In addition, the TCGA data comparing normal and tumor tissues were obtained and analyzed via UALCAN (http://ualcan.path.uab.edu/), as previously detailed.[27]

Statistical analysis

SPSS 24.0 software (IBM, Armonk, NY) was used to perform all statistical analyses and GraphPad Prism 7 was used to visualize the results. All bar plots are presented as mean (SD) or number of samples (n). Statistical comparisons were made using the chi-square (χ^2) test, Fisher's exact test, or one-way analysis of variance followed by the Student t test. A Gehan-Breslow-Wilcoxon test was used to compare survival rates between the DDR1-high and DDR1-low expressing groups of patients. $P < .05$ was considered statistically significant.

RESULTS

DDR1 expression levels in HNSCC/OSCC specimens in the database

Expression levels of DDR1 in HNSCC based on samples from UALCAN TCGA analysis ($n = 564$) are presented in Figure 1A. DDR1 expression levels were

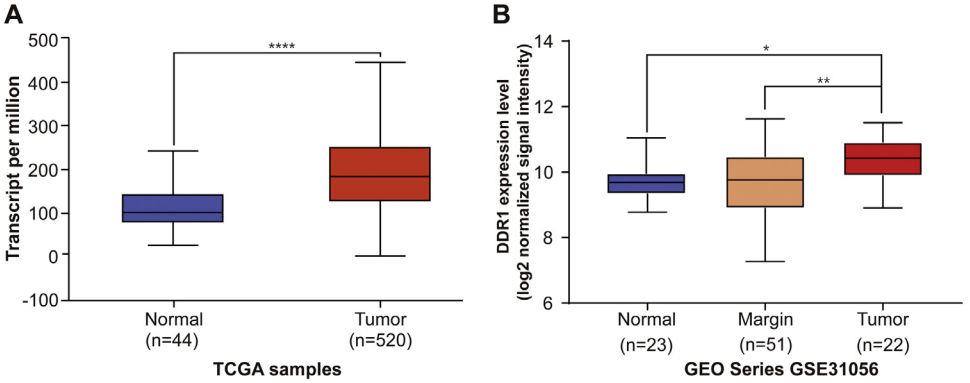

Fig. 1. Discoidin domain receptor 1 (DDR1) expression levels are higher in both head and neck squamous cell carcinoma (HNSCC) and oral squamous cell carcinoma (OSCC) tumor tissues compared to those in normal tissues or margin tissues. The 2 different series shown were taken from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) database, respectively. **(A)** Analysis of DDR1 transcripts per million (TPM) with respect to patients with HNSCC ($n = 564$) available through the UALCAN website. The data set included normal ($n = 44$) and tumor ($n = 520$) specimens. **(B)** Analysis of DDR1 expression levels based on the GSE31056 series in the GEO database ($n = 94$). The data set included normal ($n = 23$), margin ($n = 51$), and tumor ($n = 22$) tissue specimens. **(A)** Student t tests, **(B)** one-way analysis of variance, $^*P < .05$. $^{**}P < .01$. $^{***}P < .0001$.

significantly ($P < .0001$) increased in tumor samples compared to those in normal samples. Statistical analysis of the GSE31056 microarray data from the Gene Expression Omnibus database ($n = 96$ samples) was consistent with the HNSCC results, with DDR1 expression in OSCC tissues being significantly higher than that in normal tissues ($P < .05$; Figure 1B).

Correlation between DDR1 expression and survival of patients with HNSCC

To verify whether DDR1 expression was associated with patient survival, we evaluated data from 260 patients with HNSCC for which clinical, RNA-Seq, and gene mutation information was obtained using TCGA RTools. The results indicated that the independent mortality risk for patients with HNSCC with high DDR1 expression was significant compared to that for patients with HNSCC with low DDR1 expression ($P = .049$; Figure 2A). Furthermore, the survival rate was considerably different due to T-stages ($P = .0316$; Figure 2B) Further analysis revealed that numbers of gene mutation were increasing in tandem with T-stage ($P = .0010$; Figure 2C). Similar tendencies, although not confirmed as statistically significant, were observed in DDR1 expression level ($P = .0965$; Figure 2D).

Expression patterns of DDR1 in human OSCC specimens

Previous analysis of biological data indicated that DDR1 may play a role in the progression of HNSCC. To verify the role of DDR1 in OSCC, we first compared DDR1 expression levels in primary tumor

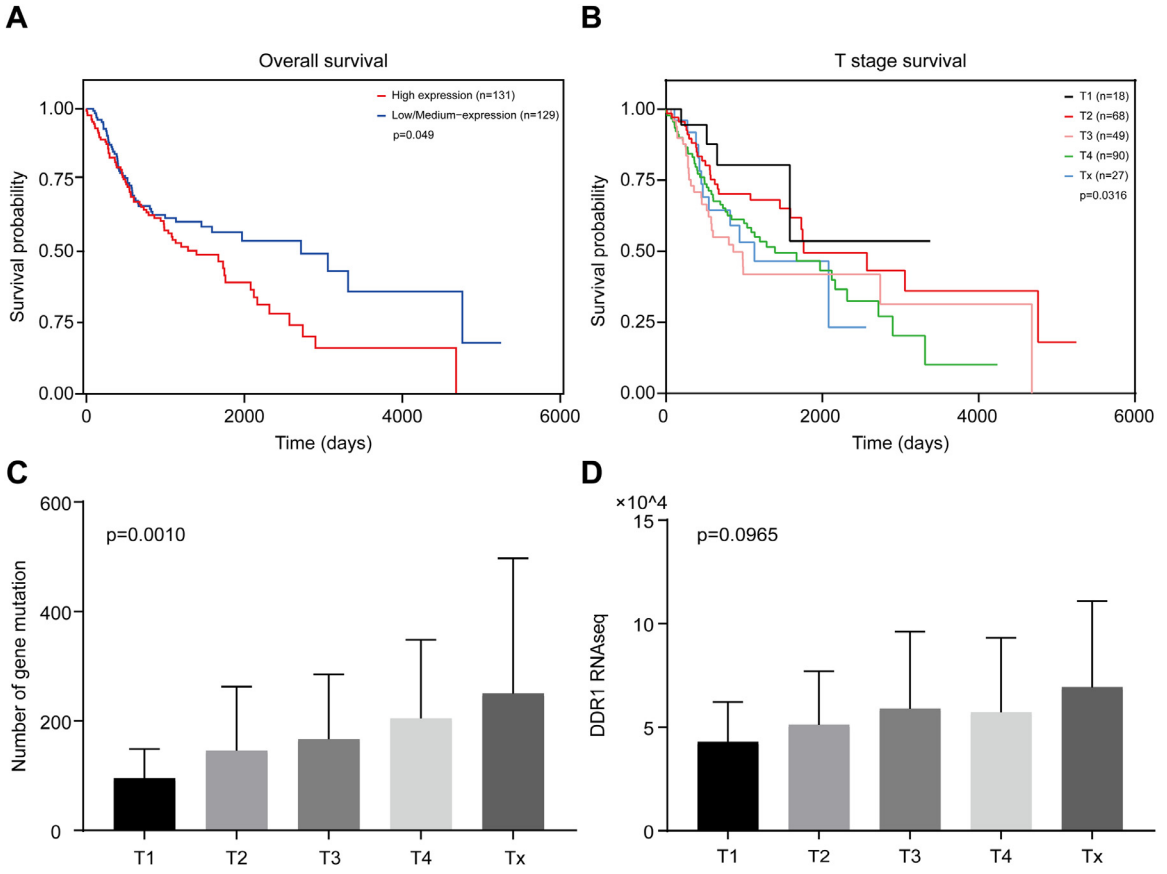

Fig. 2. Discoidin domain receptor 1 (DDR1) expression has an adverse impact on overall survival for patients with HNSCC. **(A)** Overall patient survival curves with respect to DDR1 expression level. Patients with head and neck squamous cell carcinoma (HNSCC) and survival information available in The Cancer Genome Atlas (TCGA; $n = 260$) were divided into 2 groups, DDR1 high expression ($n = 131$) and DDR1 low expression ($n = 129$), based on median DDR1 RNA sequencing data. The results showed that samples with higher DDR1 expression levels were associated with lower overall survival ($P = .049$). **(B)** T-stage survival curve for samples that included DDR1 expression data. A total of 260 samples were grouped as T1 ($n = 18$), T2 ($n = 68$), T3 ($n = 49$), T4 ($n = 90$), or Tx ($n = 27$) based on tumor pathological classification. The results indicated that survival rate was significantly related to T-stage ($P = .0316$). **(C)** The number of gene mutations increased in accordance with the increase in T-stage ($P = .0010$). **(D)** DDR1 expression levels based on RNA sequencing results showed no statistical significance with respect to overall survival ($P = .0965$). Results in **(A), (B)** and **(C), (D)** were separately tested using log-rank (Mantel-Cox) tests and one-way analysis of variance.

tissues, metastatic lymph nodes, and corresponding pericarcinomatous tissues using immunohistochemistry. Representative immunohistochemical features of the tumor tissues, metastatic lymph nodes, and pericarcinomatous tissues are shown in Figures 3A to 3C. DDR1 expression levels observed in tumor tissues and metastatic lymph nodes were significantly increased compared to that in pericarcinomatous tissues ($P < .005$; Figure 3D). Figure 3E shows the relative levels of the negative and positive controls. There was no significant difference in DDR1 expression levels between tumor tissues and metastatic lymph nodes.

DDR1 positively correlated with OSCC T-stage classification and overall survival

Immunohistochemistry staining results were further analyzed to determine the potential connection between DDR1 expression and the demographic and clinicopathologic characteristics of the 34 patients with OSCC. Figures 4A and 4B show that the expression level of DDR1 positively correlated to OSCC T-stage classification. Specifically, DDR1 expression increased accordingly with higher T-stage classification of OSCC tumors. In contrast, there was no significant correlation between DDR1 expression and patient gender, age, N-stage classification, lymph node metastasis, or neoplasm recurrence (Table I).

We further evaluated the effect of DDR1 expression during follow-up of the 34 patients with OSCC. The patients were divided into 2 groups (low/medium and high) according to DDR1 expression level. Figure 4C shows the representative examples of the high-expression DDR1 group and low/medium-expression DDR1 group classified by the median immunohistochemistry (IHC) score. Overall survival rates were calculated using the Kaplan-Meier method. As shown in Figure 4D, patients in the high-expression DDR1 group tended to have a worse survival curve ($P = .0293$) compared to those in the low/medium-expression DDR1 group. These findings were in accordance with the bioinformatic analysis of patients with HNSCC.

Inhibition of DDR1 reduces proliferation in OSCC cells

To detect expression of DDR1 in OSCC cells, western blotting was performed by exposing CAL-27 and SCC-

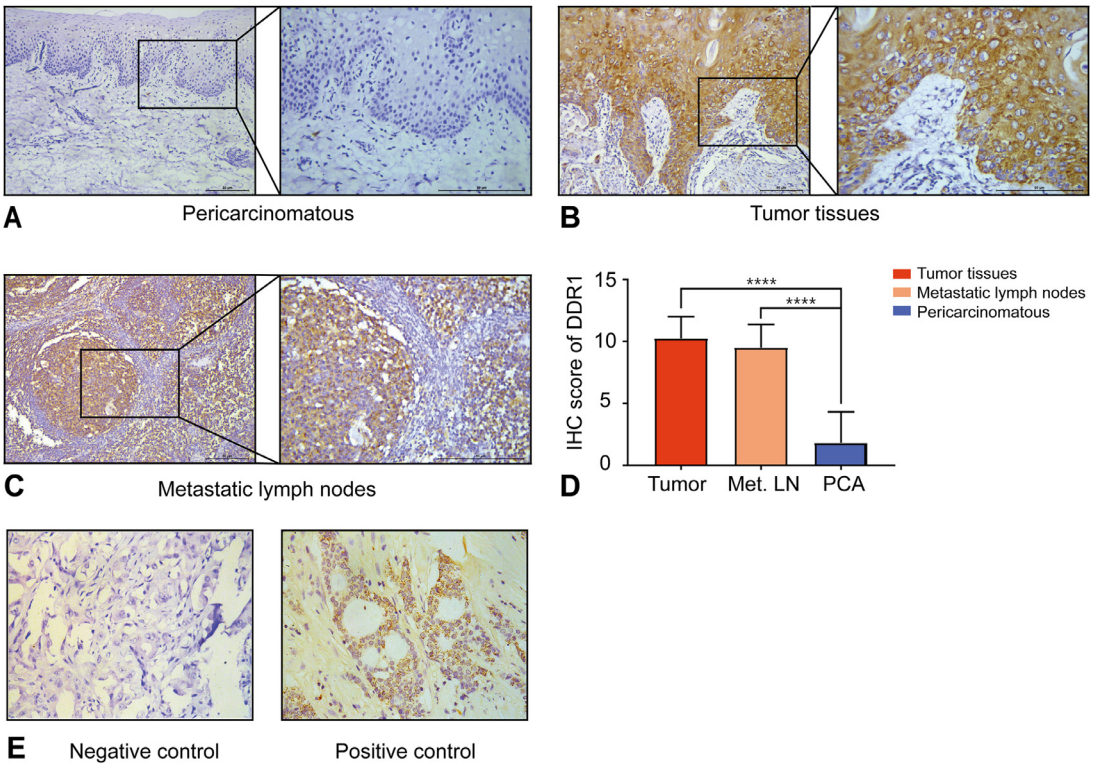

Fig. 3. DDR1 expression was significantly increased in oral squamous cell carcinoma (OSCC tumor tissue and metastatic lymph node tissue compared to that in pericarcinomatous tissue. **(A)** Staining was hardly observed in pericarcinomatous cells. **(B)** and **(C)** Strong staining for DDR1 was clearly detected in oral tumor specimens and metastatic tissues. The staining was primarily observed in the cytomembrane and cytoplasm. **(D)** Immunoreactive scores for DDR1 staining. Results are shown as mean (SD). Analysis of variance, $***P < .0001$; $n = 34$. **(E)** Negative control from OSCC specimen and positive control from breast carcinoma specimen.

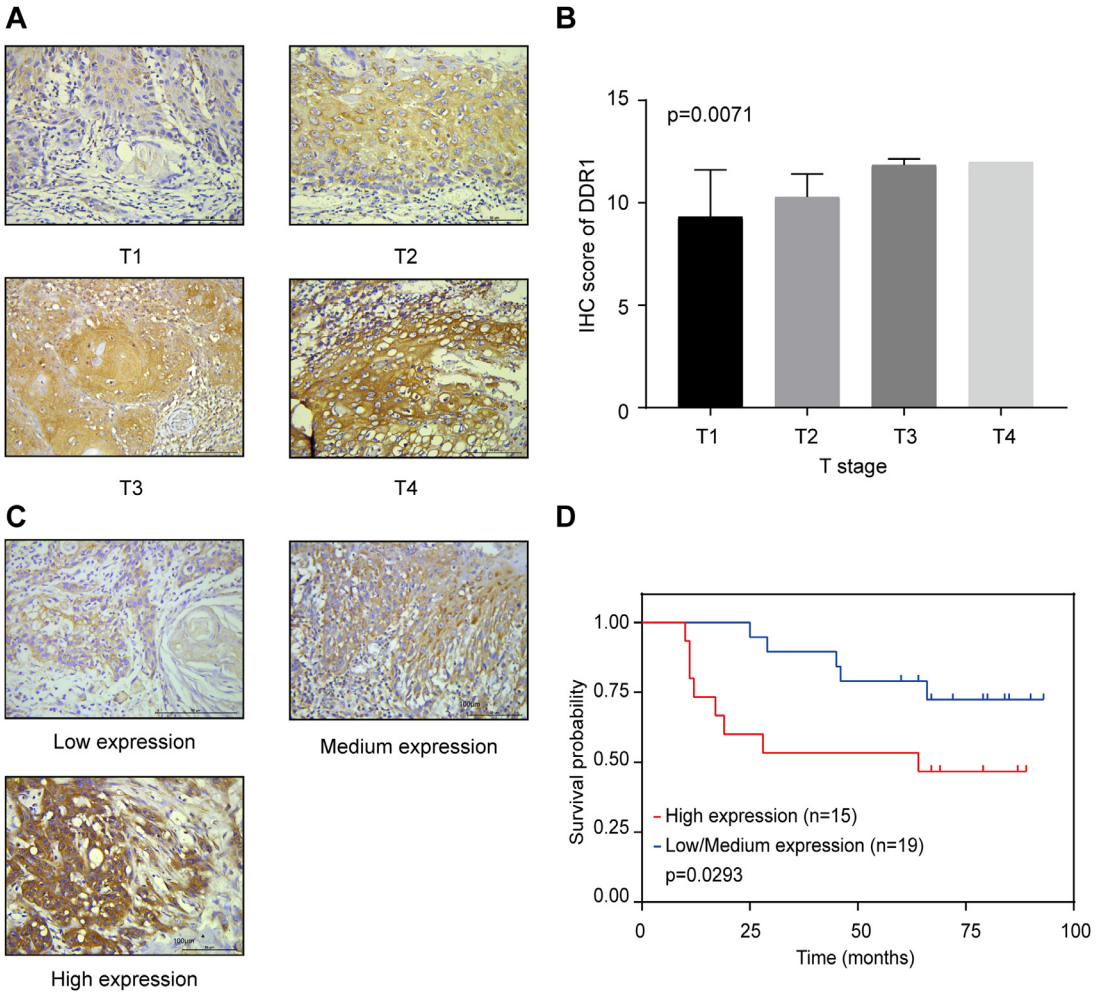

Fig. 4. Increased discoidin domain receptor 1 (DDR1) expression levels in patients with oral squamous cell carcinoma (OSCC) paralleled increased T-stage and had an adverse effect on patient overall survival. **(A)** Gradually increasing staining intensity and increasing percentage of positive cells could be distinctly observed from stages T1 to T4, and increasing staining intensity could also be observed in the cytoplasm and part of the nucleus. **(B)** Analysis of DDR1 expression relative to T-stage (n = 34). DDR1 expression was correlated with T1 tumors (n = 12), T2 tumors (n = 16), T3 tumors (n = 4), and T4 tumors (n = 2) and was found to be significant (one-way analysis of variance, P = .0071). **(C)** Typical pathological characteristics of high/medium/low expression level of DDR1. The median Immunohistochemistry score was used as the criteria of high/medium/low distribution. **(D)** Overall survival curves for patients with OSCC who exhibited high expression of DDR1 (n = 15) and low/medium expression of DDR1 (n = 19) based on median DDR1 immunohistochemical staining score. Gehan-Breslow-Wilcoxon (Mantel-Cox) test, P = .0293.

4 cells to different concentrations of TKI-nilotinib. As shown in Figure 5A, protein expression level was decreased in nilotinib-exposed groups compared to the control group ($P < .05$). To further investigate the function of DDR1 in OSCC cells, we conducted CCK8 and colony formation assays with OSCC cells grouped by incremental nilotinib concentration (0, 1, 2, and 4 μg/μL). Decreased cell viability in response to increasing nilotinib concentration was observed, as shown in Figure 5B ($P < .05$), except for the 1 μg/μL nilotinib group. In addition, a tendency toward a lower ratio of colony formation was seen in 1, 2, 4 μg/μL nilotinib concentrations (Figure 5C; $P < .01$).

DISCUSSION

Increased DDR1 expression has been substantiated by previous studies via its participation in tumor proliferation, invasion, and metastasis in breast cancer, hepatocellular carcinoma, thyroid cancer, and lung cancer.[15,16,28,29] However, the existence and possible function of DDR1 in OSCC tumor cells remain unknown. In the present study, we demonstrated through the use of bioinformatical and functional methods that higher DDR1 expression levels in OSCC was related to tumor progression and correlated with prognosis.

For validation purposes, 2 series of microarray data from different platforms were evaluated for HNSCC and

Table I. Clinicopathological features of patients with OSCC and their association with DDR1 expression in OSCC

Characteristics	*n*	*DDR1 expression*		χ^2 *P*
		Low/medium	*High*	
Total	34	19	15	
Gender				
Female	5	2	3	
Male	29	17	12	.634
Age				
<50	17	9	8	
≥50	17	10	7	1.000
N classification				
N0	10	6	4	
N1-N3	24	13	11	1.000
T classification				
T1-T2	28	19	9	
T3-T4	6	0	6	.004
Histologic stage				
Well	17	10	7	
Moderate, poor	17	9	8	1.000
Recurrence				
Yes	6	5	1	
No	28	14	14	.196
Survival status				
Live	21	14	7	
Dead	13	5	8	.160

OSCC, oral squamous cell carcinoma; *DDR1*, discoidin domain receptor 1.

OSCC specimens. DDR1 gene expression was higher in tumor tissues compared to that in normal tissues. Moreover, using immunohistochemical staining and statistical analysis, we further determined high DDR1 expression at the protein level in OSCC specimens. A pronounced, strong positive straining was observed in the cell membrane and cytoplasm of neoplastic epithelial cells. Our findings regarding DDR1 expression are aligned with those previously reported by Chou et al.[30]

Another finding highlighted in our study was the association between DDR1 expression and OSCC clinical features, such as sex, age, tumor-node-metastasis classification, tumor recurrence, and survival rate. In the preliminary analysis of TCGA data, we found that higher DDR1 expression was overall correlated with negative effects on patients with HNSCC. To be specific, higher DDR1 expression tend to be related to higher T-stage and may lead to poorer prognosis. Our results were consistent with previous studies demonstrating that high expression of DDR1 corelates with a poor survival prognosis for patients with non-small cell lung carcinomas,[31] pancreatic ductal adenocarcinoma,[32] and renal cell carcinoma.[33] Furthermore, in individuals who expressed DDR1, T-stage survival was significantly dependent on the T-stage classification. This was possibly due to the proportion of cells with the gene mutation increasing as the tumor volume and the distance of adjacent tissue involvement increased, because the expression level of DDR1 (represented by RNA-Seq) was not statistically significantly different, even though it did exhibit a similar trend. In addition, our data from the follow-up of patients with OSCC collected during the final 8 years of the study showed that the effect of DDR1 on patient survival was in accordance with that in patients with HNSCC. Regardless, based on the IHC score, DDR1 expression increased with higher T-stage for OSCC, which differed from that of HNSCC. There are 2 potential reasons that may explain this difference. First, RNA-Seq helps quantify expression at the gene or transcript level but does not directly measure protein expression. Therefore, our results from the TCGA RNA-Seq analysis may vary from those based on IHC scores. Second, so far, few studies on DDR1 expression have concentrated on OSCC[30] and thus there is the need to further investigate whether DDR1 expression is related to T-stage in other HNSCCs. We also demonstrated in the present study that DDR1 in the OSCC microenvironment did not correlate to patient sex, age, N-stage classification, histologic stage, or recurrence. These findings suggest that DDR1 may be more important in OSCC tumor cell proliferation and invasion than in metastasis. Further studies in larger populations need to be performed to confirm these findings.

This study also pinpoints the existence of DDR1 in OSCC cells and its probable function in cell proliferation, which agrees with the analysis of OSCC bioinformation and clinical research above. Nilotinib is among the second-generation TKIs approved by the US Food and Drug Administration for the treatment of chronic myelogenous leukemia[18] and has also been utilized in the treatment of other cancers such as colorectal cancer.[34] It has been proved chemically by Rix et al. that DDR1 was the highest interactor of nilotinib with 15 unique peptides and 17% sequence coverage.[35] Our study adds to the accumulating evidence that suggests that DDR1 is highly expressed in OSCC cells and takes part in cell proliferation. This effect can be suppressed by nilotinib dependent on concentration, because cell viability and cell colony formation decline after exposure to nilotinib. In recent studies, knockdown of DDR1 has been shown to significantly decrease the survival of collagen-treated L428 Hodgkin lymphoma cells[36] and activated DDR1 increases Reed-Sternberg cell survival in vitro.[37] In vivo, Sun et al. reported that DDR1-knockout mice grow less robustly than wild-type mice.[38] This function may be performed through extracellular matrix remodeling because the tumor-associated stroma in DDR1-knockout mice exhibits less collagen deposition compared to that in wild-type mice. Lai et al. also proved that collagen could mediate

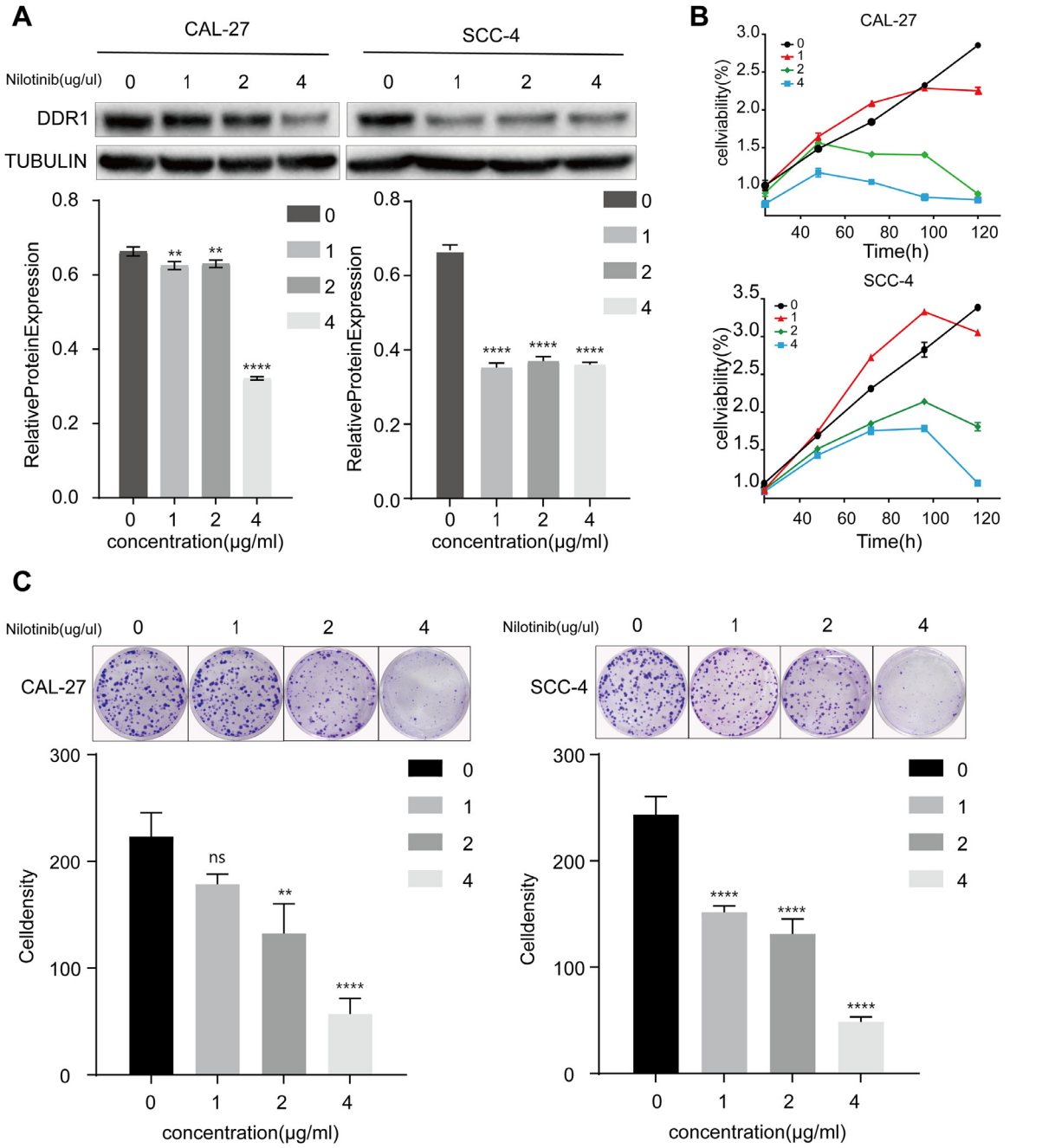

Fig. 5. A considerable amount of DDR1 was detected in oral cancer cell lines and could be suppressed by tyrosine kinase inhibitors. **(A)** DDR1 is readily detectable in OSCC cell lines CAL-27 and SCC-4 using western blotting, and its expression decreased with increasing nilotinib concentration after a 24 h-exposure. **(B)** Growth rates of CAL-27 and SCC-4 cells measured using the Cell Counting Kit-8 assay following exposure to 0, 1, 2, 4 μg/μL of nilotinib and, as shown, 2 and 4 μg/μL of nilotinib better suppressed cell proliferation compared with 0 and 4 μg/μL. Two-way analysis of variance, $P < .05$. **(C)** Colony formation assay after exposure to nilotinib in CAL-27 and SCC-4 cells for 7 days; the colony formation ability was inhibited as nilotinib concentration increased. The mean number of colonies for each well was determined using three independent assays. **(A), (C)**: One-way analysis of variance, $^{*}P < 0.05$. $^{**}P < 0.01$. $^{***}P < 0.0001$.

HNSCC tumor proliferation via DDR1. By knocking down DDR1 in HNSCC cell lines, the tumor-promoting effects of collagen were clearly demonstrated, although the underlying mechanisms were unclear.[39] Moreover, a recent study reported that DDR1 increased the transcription of collagen IV, which is the primary upregulated collagen in OSCC and fibrosis disease, via nuclear translocation.[40] Interestingly, Chen et al. recently reported that in betel quid−associated OSCC tissues, DDR1 was positively related to collagen IV in

the process of basement membrane deposition, suggesting that DDR1 and collagen were crucial drivers of tumor cell growth and invasion.[41] Because collagen disorders are common in pathologic tissues including tumor tissues, collagen accumulation may be a key driver of DDR1 activation. Conversely, DDR1 can also mediate collagen transcription to participate in extracellular matrix remodeling. This interaction between DDR1 and collagen may be one of the probable reasons for DDR1's pivotal role in tumor development. Furthermore, Azizi et al. identified DDR1 as an important target for modulating epithelial-mesenchymal transition in prostate cancer cell lines.[42] DDR1 inhibition can prevent epithelial-mesenchymal transition by inhibiting Pyk2 and MKK7 signaling pathways. However, the precise molecular mechanism regarding the effect of DDR1 on tumor proliferation and invasion remains unclear; thus, more research is still required before the final goal can be achieved.

Based on the discussion above, it can be concluded that DDR1 may be a novel biomarker for OSCC, and possibly even for HNSCC. Our findings will facilitate the next step in determining the function of DDR1 in promoting tumor growth. Further studies on the molecular mechanisms involved are needed to elucidate the biological role of DDR1 in the pathogenesis of OSCC, in vivo or in vitro. This information will be essential to bridge the gap that exists between our current understanding and that needed to develop DDR1-targeted therapies for OSCC.

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