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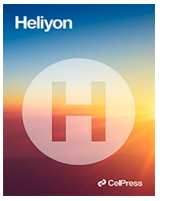

Research article

ANKRD2 expression combined with TNFRSF19 expression for evaluating the prognosis of oral squamous cell carcinoma patients

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ABSTRACT

Objective: As an important chemotherapy drug, cisplatin has been widely used in the treatment of many cancers. However, many patients, including oral squamous cell carcinoma (OSCC) patients, experience unacceptable outcomes from cisplatin treatment. Thus, we devised a risk model for predicting the sensitivity of OSCC patients to cisplatin treatment, to provide a reference for clinical practice.

Methods: CAL-27 and SCC-9 cell lines treated or not with cisplatin and data from The Cancer Genome Atlas (TCGA) were screened for simultaneously and significantly differentially expressed genes. Next, we built a risk model for predicting cisplatin sensitivity in OSCC patients. Reverse transcription-polymerase chain reaction (RT-PCR), pathological samples and clinical data were used to examine the reliability of the model.

Results: ANKRD2 and TNFRSF19 were differentially expressed between the OSCC metastasis cell line HSC-3 treated and not treated with cisplatin, as well as between the OSCC cell line SCC-25 and the cell line SCC25-DDP, which has cisplatin chemoresistance. We found that the expression of ANKRD2 and TNFRSF19 had a significant influence on the prognosis of OSCC patients. The risk model that combined ANKRD2 and TNFRSF19 to predict sensitivity to cisplatin in OSCC patients was confirmed by analysing the pathological samples and follow-up information of clinical patients.

Conclusions: The expression of ANKRD2 and TNFRSF19 is associated with cisplatin sensitivity and prognosis in patients with OSCC. The survival outcome of patients with oral squamous cell carcinoma (OSCC) was found to be significantly worse in those with high expression of ANKRD2 combined with low expression of TNFRSF19. ANKRD2 and TNFRSF19 may be targets for cisplatin sensitivity prediction in OSCC patients. These findings may provide novel strategies for overcoming cisplatin resistance.

1. Introduction

Oral squamous cell carcinoma is one of the most prevalent cancers throughout the world [1]. Currently, many methods, such as surgery, radiotherapy, and chemotherapy, have been used to treat OSCC [2], but the prognosis is still unsatisfactory [3], and the five-year survival rate remains under 50 % [4]. Although cisplatin, as a crucial chemotherapy drug, has been widely used in cancer treatment [5], its outcomes of treatment of OSCC patients is still unsatisfactory. Many patients experience cisplatin chemoresistance

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[6] and ultimately exhibit a worse prognosis. The problem of cisplatin chemoresistance, has been the subject of several large studies. For example, Shi et al. demonstrated that the expression level of HNRNPU can influence chemosensitivity in bladder carcinoma cells via NF1 regulation [7]. Berkel et al. found that higher expression of estrogen or estrogen receptors is generally associated with higher cisplatin resistance in cancer in vitro [8].

In head and neck cancer area, scholars have discovered multiple mechanisms of cisplatin chemoresistance including cancer stem cells, autophagy, epithelial-mesenchymal transition, drug efflux, and metabolic reprogramming [9]. Xu et al. found that the cisplatin resistance-regulating roles of the TNFAIP2/KEAP1/NRF2/JNK axis in HNSCC, suggesting that TNFAIP2 might be a potential target of HNSCC cisplatin resistance [10]. Osman et al. demonstrated that cisplatin resistance and development of distant metastasis are associated with dysregulated and epigenetically reprogrammed KEAP1-NRF2 signaling pathway [11]. Although many discoveries have emerged concerning the reason for cisplatin resistance in oral cancer patients [12], the mechanism is still not clearly understood. If the sensitivity of patients to cisplatin chemotherapy can be predicted in advance and if their treatment method can be immediately changed, the survival rate of patients is expected to improve. Currently, the genetic sequencing landscape has seen extensive applications in cancer studies [13], including in head and neck cancer [14]. To identify patterns between patient characteristics and survival, scientists have used multiple methods to develop several risk evaluation models for patients in an effort to predict the prognosis of patients [15], which have helped to develop individualized treatment plans to improve treatment efficiency [16]. Howard et al. developed a machine learning model which can identify patients with intermediate-risk head and neck squamous cell carcinoma who would benefit from chemoradiation [17]. Chen et al. constructed a signature of twelve m6A regulator-related genes using the Cox risk model, combined with the least absolute shrinkage and selection operator variable screening algorithm to predict the responses and prognoses of immune checkpoint inhibitor during treatment [18].

Herein, we designed a risk evaluation model through database mining and the collection of clinical information. Based on these data, we strived to detect potential targets of cisplatin sensitivity in OSCC and provide a new strategy for choosing OSCC chemotherapy.

2. Materials and methods

2.1. Cell culture and treatment with cisplatin

Human oral squamous carcinoma cell lines, including CAL-27, SCC-9, and HSC-3 were purchased from the American Type Culture Collection (ATCC). The human oral squamous carcinoma cell line SCC-25 and human oral squamous carcinoma cisplatin resistant cell line SCC25-DDP were purchased from Jinyuan Biotechnology (SRCC, China). All the cell lines were validated by short tandem repeat profiling analysis and were free of mycoplasma contamination. CAL-27, SCC-25, and SCC25-DDP cells were cultured in DMEM supplemented with 10 % FBS, and SCC-9 cells were cultured in DMEM/F-12 supplemented with 10 % FBS. They were all grown in a 37 °C humidified incubator containing 5 % CO_2.

The HSC-3, CAL-27 and SCC-9 cell lines were divided into control and cisplatin-treated groups. Cells in the cisplatin-treated groups were treated with 5 μM/ml cisplatin according to previous study [19]. Twenty-four hours later, RNA was extracted, and RNA of cells in the cell lines from CAL-27 and SCC-9 cells treated with and without cisplatin was sent for transcriptome sequencing (IGE Biotechnology, China).

2.2. RNA extraction, real-time quantitative RT–PCR, and RNA sequencing

RNA was extracted from cells treated with or without cisplatin by an RNA purification kit according to the manufacturer's instructions (EZBioscience, USA) and then reverse transcribed into cDNA by using HiScript III RT SuperMix for qPCR (+gDNA wiper) following the manufacturer's instructions (Vazyme, China). Primers (ANKRD2 forward: 5′-CTGCGGAAGAAACGCAAGC-3′, reversed: 3′-AGGGCCAGTGATCTCCTCG-5'. TNFRSF19 forward： 5′-GACCTCAGCTCCACGAATATG-3′, reversed: 3′-CACCCCACAACCAAGAGTCG-5′), 1 μl cDNA, and 2xChamQ Universal SYBR qPCR Master Mix were mixed in 10 μl reaction volume. The relative expression of mRNA was detected by the Roche LightCycler 480 II Real-time PCR machine (Roche, USA).

2.3. Detection from database

In the CAL-27 cell line, which was treated with or without cisplatin and sequenced by using 16,505 genomic DNA probes, 14,412 genes were detected. In the cell line SCC-9, treated with or without cisplatin and sequenced via 16,847 genomic DNA probes, 14,568 genes were detected.

We downloaded the data of 292 samples, including 19 normal tissue samples and 273 oral squamous cell carcinoma samples from The Cancer Genome Atlas Program (TCGA, https://cibersortx.stanford.edu/). By using the "edgeR" package [20] to identify the DEGs with $|\log2FC| \geq 2$ and $P < 0.01$, gene sequences were analysed for differential expression.

2.4. Data filtration

CAL-27 cell line sequence data and SCC-9 cell line sequence data with and without cisplatin treatment were selected by $|\log2FC| \geq 2$ and $P < 0.01$. Three of groups had interactions to detect significant differentially expressed genes in the three data groups. Afterwards, the Pearson correlation test was performed on the selected genes by using the "Corrplot" package (GitHub - taiyun/corrplot: A

visual exploratory tool on correlation matrix).

2.5. Development of a risk prediction model

Multivariate Cox regression by the "Survival" package (GitHub - therneau/survival: Survival package for R) was used to identify some genes that seemed to significantly influence the prognosis of patients. The Kaplan–Meier method helped us to verify that the survival of patients with different expression levels of the selected genes was different. After filtering, we built a nomogram model that could predict the prognosis of patients with OSCC [21].

2.6. Clinical data

From 2015 to 2018, 117 oral squamous cell carcinoma patients were accepted for surgical treatment and post-surgery cisplatin chemotherapy in the Department of Stomatology, Second Affiliate Hospital of Shantou University. In frozen tissue sections taken from surgery, the boundary of the section had approached the normal tissue. Tumour tissues were diagnosed via pathological examinations by the Department of Pathology, Second Affiliate Hospital of Shantou University. Clinical staging were performed following the 8th Edition TNM Classification for Head and Neck Cancer developed by the Union for International Cancer Control (UICC) and the American Joint Committee on Cancer (AJCC). Access to patient survival information was collected by telephone contact.

2.7. Immunohistochemistry

Pathological tissue sections were obtained from each patient for immunohistochemical staining. The immunohistochemistry procedure followed standard protocols [19]. After deparaffinization, antigen retrieval was conducted using Tris-EDTA buffer (#C1038, Solarbio) in a microwave oven for 15 min. Briefly, the tissue sections were blocked sequentially with 3% H_2O_2 and normal serum and then incubated with primary antibodies (#AF723 and # NBP1-91670, Biotechne, USA) at 4°C overnight. Tissues were incubated with a biotinylated secondary antibody and conjugated with a streptavidin-HRP complex (ready-to-use SP kit; Zhongshan

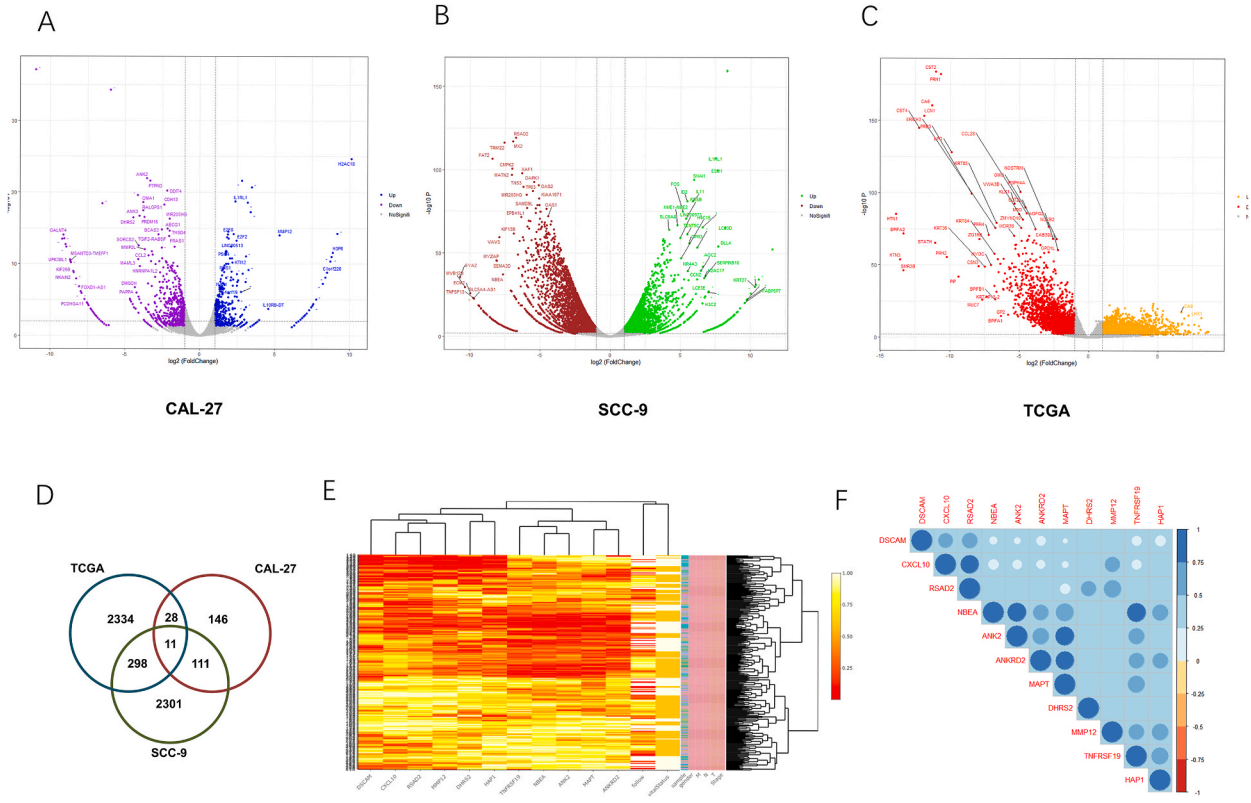

Fig. 1. Eleven significant differentially expressed genes and their correlation analysis A-C) Volcano plots depicting differential gene expression in the CAL-27 and SCC-9 cell lines treated with or without cisplatin, as well as in TCGA OSCC samples compared to normal tissues. D) Venn diagram shows that 11 genes were differentially expressed between the two subsets of all three groups simultaneously. E) Eleven genes had significant differential expression levels in 272 head and neck squamous cell carcinoma cases. F) The correlation among the 11 genes in the tested samples exhibited a high degree of complexity.

Co., China). Finally, the sections were visualized with 3-3′-diaminobenzidine, counterstained with haematoxylin and mounted. The samples were rinsed with PBS between each step.

2.8. Evaluation of immunohistochemistry

The tissues were evaluated by two pathologists who assessed the number of positive cells and the staining intensity. Positive staining was assessed using a semiquantitative scoring system. The percentage of positive cells was scored as follows: 0 (no staining), 1 (<1/3 staining), 2 (1/3 to 2/3 staining), and 3 (>2/3 staining). Staining intensity was scored as follows:0 (negative), 1 (weak positive), 2 (medium positive), and 3 (strong positive). The final evaluation of protein expression was based on the sum of the two scores: a score of 0–2 was defined as low expression, while a score>2 was defined as high expression.

2.9. Statistical analyses

All of the statistical analyses were done in R software (v4.1.2). The survival analysis used the Kaplan–Meier algorithm. The significant difference in the survival curve was analysed by using the log-rank test. Multivariate Cox regression was used to specify the candidate genes. One-way analysis of variance (one-way ANOVA) and the chi-square test were used for correlation analysis.

3. Results

3.1. Eleven significant differentially expressed genes were obtained

After database selection by $|\log2FC| \geq 2$ and $P < 0.01$, the CAL-27 cell line showed 296 significantly expressed genes, and the SCC-9 cell line 2721 between their cisplatin- and non-cisplatin-treated groups (Fig. 1 A B). Expression of 55270 genes were detected in the 292 oral squamous cell carcinoma samples download from TCGA database, including 19 normal tissue samples and 273 tumor samples. After differential analysis by using the edgeR package, 2671 differentially expressed genes were obtained in the TCGA samples restricted by $|\log2FC| \geq 2$ and $P < 0.01$ (Fig. 1 C). Among them, 28 differentially expressed genes coexisted in the TCGA

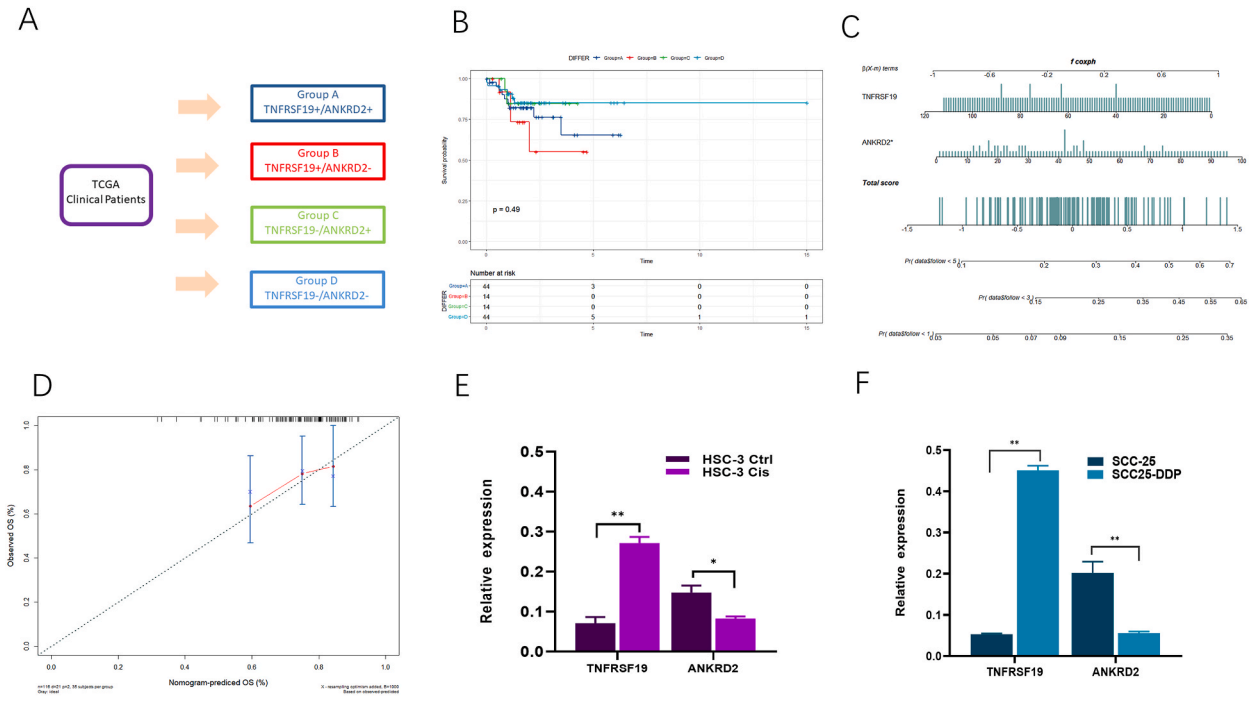

Fig. 2. Designed and validated prediction model. A) TCGA patients were divided into four groups: Group A: high expression of TNFRSF19 and ANKRD2; Group B: high expression of TNFRSF19 and low expression of ANKRD2; Group C: low expression of TNFRSF19 and high expression of ANKRD2; Group D: low expression of TNFRSF19 and ANKRD2. B) The Kaplan-Meier analysis revealed that Group B exhibited the most unfavorable survival prognosis. C) The nomogram demonstrates that the prognostication of one-year, three-year, and five-year outcomes in patients with oral squamous cell carcinoma (OSCC) can be achieved through the assessment of distinct expression levels of TNFRSF19 and ANKRD2. D) Calibration demonstrated the accuracy of the risk model. The expression level of TNFRSF19 in E) HSC-3 cells treated with cisplatin was significantly higher than in the same cells treated with cisplatin. ANKRD2 has the opposite tendency. F) SCC25-DPP, a cisplatin-resistant cell line of OSCC, showed higher expression of TNFRSF19 but lower expression of ANKRD2 than SCC25. (*$P < 0.05$; **$P < 0.01$.)

sample group and CAL-27 group, 298 differentially expressed pseudogenes coexisted in the TCGA sample group and SCC-9 group, and 111 differentially expressed genes coexisted in the CAL-27 group and SCC-9 group (Fig. 1 D). Eleven differentially expressed genes were simultaneously found in all three groups (DSCAM, CXCL10, RSAD2, MMP12, DHRS2, HAP1, TNFRSF19, NBEA, ANK2, MAPT, and ANKRD2) (Fig. 1E).

3.2. Complex correlations emerged between 11 genes

To calculate the correlations between genes' expression levels and the probable influence of each gene on the others, we selected the "corrplot" package to perform the correlation analysis, and the results were presented a correlation heatmap. A darker blue colour of spots between two genes that meet in a given row and column indicates that the correlation coefficient between gene's expression was closer to 1, meaning a positive correlation. While a colour closer to red denotes a correlation closer to −1, indicating negative correlation between the two genes (Fig. 1F). For example, the correlation between NBEA and ANK2 shows a significantly positive correlation, and a strong positive correlation also occurs between CXCL10 and RSAD2, NBEA and TNFRSF19, and ANK2 and WAPT. These results suggests that multiple genes may cooperate to regulate cancer activity.

3.3. ANKRD2 and TNFRSF19 influence the prognosis of OSCC patients

Both CAL-27 and SCC-9 cells exhibited differential expression of multiple genes when treated with vs. without cisplatin. There were also expression differences between tumour and normal samples in the TCGA database. We expect these genes become an important target influencing the prognosis of OSCC patients.

To confirm our inference, we connected the data with clinical information from samples of TCGA. After the evaluation of the multivariate COX regression by using the "Survival" package, we found that TNFRSRF19 ($P = 0.027$) and ANKRD2 ($P = 0.0445$) had a significant impact on the prognosis of OSCC patients (Supplemental Table 1). Based on the median score, genes were divided into high expression and low expression groups. The patients were divided into four groups: Group A included patients with high expression of both ANKRD2 and TNFRSF19. Patients expressing a high level of TNFRSF19 but a low level of ANKRD2 were classified as Group B. Patients with a low expression of TNFRSF19 but a high expression of ANKRD2 were categorized as Group C. Patients with low expression of both genes were categorized as Group D (Fig. 2A).

3.4. The downregulation of ANKRD2 and the upregulation of TNFRSF19 are associated with a poorer prognosis in patients with OSCC

Again we used the "Survival" package for the Kaplan–Meier test. We found that patients in Group B had the worst prognosis (Fig. 2B), even if the number of samples was not large. This phenomenon intrigued us, and we decided to investigate it further. According to the results of the multivariate Cox regression, we built a nomogram for predicting the prognosis of OSCC patients (Fig. 2C). In this model, a higher expression level of ANKRD2 corresponded to a higher score, as well as a higher one-year, three-year, and five-year survival probability. In contrast, a higher expression of TNFRSF19 was associated with a lower score and lower one-year, three-year, and five-year survival rates. The bootstrapping cross-validation method was used to calculate 95 % confidence intervals, and the accuracy of the model was also verified in the calibration curve [22] (Fig. 2D).

3.5. The upregulation of TNFRSF19 and the downregulation of ANKRD2 are present in both metastatic and cisplatin-resistant OSCC cells

To examine whether the expression changes were related to sensitivity to cisplatin in OSCC cells, we used the OSCC cell lines HSC-3, SCC-25, and SCC25-DDP to test the expression levels of TNFRSF19 and ANKRD2. Compared with the control group, higher expression of TNFRSF19 but lower expression of ANKRD2 was found in HSC-3, which is the metastastic cell line of OSCC after treatment with cisplatin for 24 hours (Fig. 2E). The expression of TNFRSF19 in SCC25-DDP, and OSCC cell line with cisplatin resistance, was higher but that of ANKRD2 was lower than in SCC-25 (Fig. 2F). Similar gene expression results in metastatic and cisplatin resistant cell lines were consistent with our model predicting that higher TNFRSF19 but lower ANKRD2 expression may represent a worse prognosis of OSCC patients. Tumour metastasis and chemotherapy have been associated with a worse prognosis [23], so we wondered whether changes in the expression of these targets affected the prognosis of OSCC patients.

Therefore, we investigated further whether high expression of TNFRSF19 and low expression of ANKRD2 affected the survival or prognosis of patients with OSCC.

3.6. Clinical information collection and immunohistochemistry

To verify the reliability of the risk model, we collected follow-up information from patients and performed immunohistochemical experiments on tumour samples resected via surgery. From 2015 to 2018, 117 patients with oral squamous cell carcinoma were admitted to the Department of Stomatology, Second Affiliated Hospital of Shantou University for surgical treatment and post-surgery cisplatin chemotherapy. In order to evaluate the expression levels of TNFRSF19 and ANKRD2, we collected tumor tissue and adjacent normal tissue from patients during the surgery for immunohistochemistry experiment.

After evaluations by pathologists, the results of immunohistochemistry for TNFRSF19 and ANKRD2 were divided into high expression and low expression groups according to our evaluation protocol (Fig. 3A and B). Through this protocol, 64 patients had high expression of TNFRSF19, 53 patients had low expression of TNFRSF19, 58 patients had high expression of ANKRD2, and 59 patients

had low expression of ANKRD2. Twenty-one patients had high expression of TNFRSF19 along with high expression of ANKRD2. Forty-three patients had high expression of TNFRSF19 but low ANKRD2 expression. Thirty-seven patients had low expression of TNFRSF19 but high expression of ANKRD2. Only 16 patients presented low expression of both genes (Table 1).

We also collected other clinical information, including sex, age, clinical stage, differentiation of tumour, metastasis, and the position of the primary tumour. Patients were followed up by telephone, and their follow-up information was used for the statistical analysis.

3.7. ANKRD2 and TNFRSF19 may become potential targets that influence the prognosis of OSCC patients

According to the follow-up information, the results of the Kaplan–Meier test and the results of immunohistochemistry, the

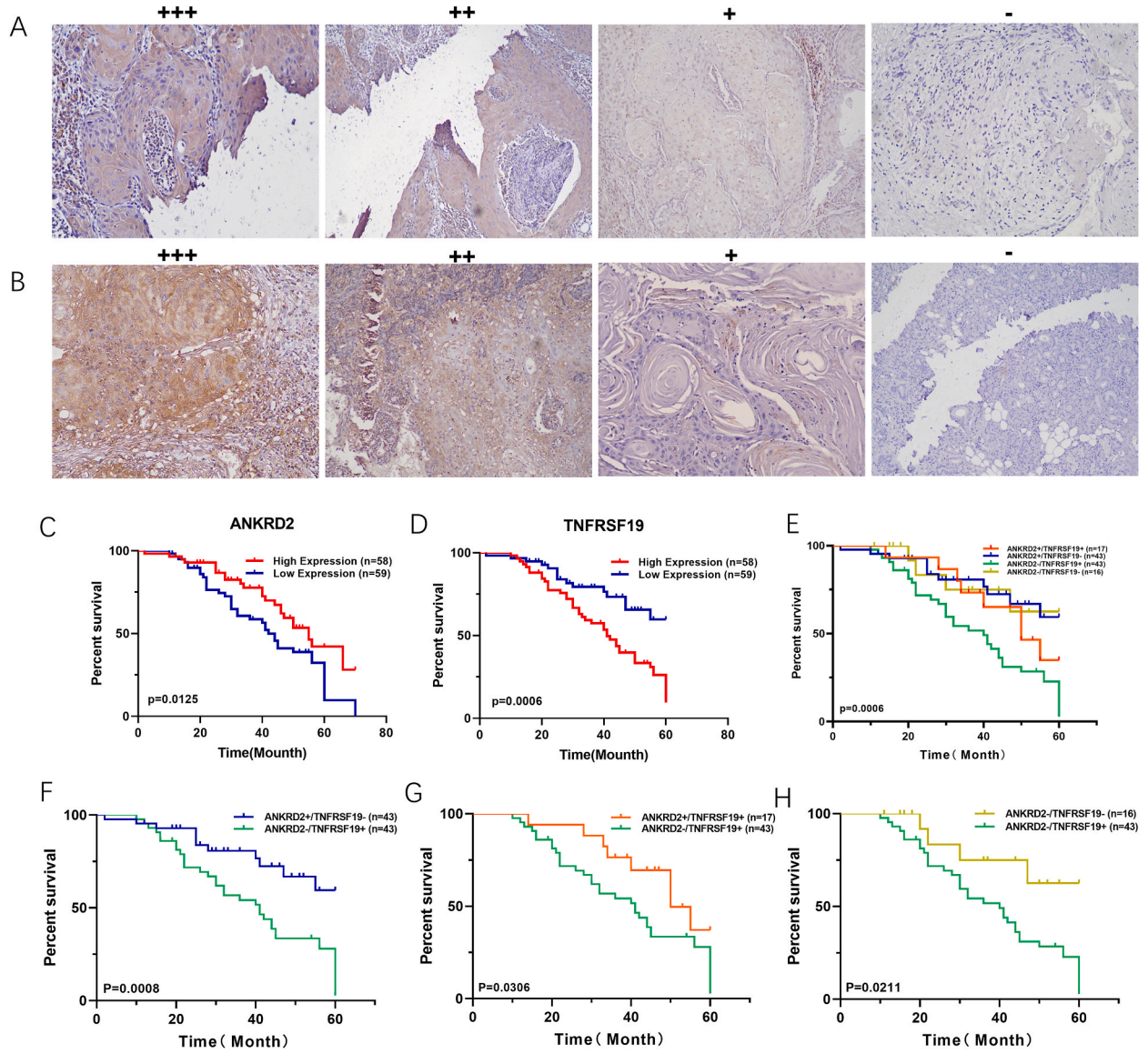

Fig. 3. Clinical patient tissue sections: A: Representative results of ANKRD2 expression in clinical patients were strongly positive (+++), moderately positive (++), weakly positive (+) and negative (−).
B: The representative results of TNFRSF19 expression in clinical patients were strongly positive (+++), moderately positive (++), weakly positive (+) and negative (−).
After statistical analysis: C-D) The high expression of ANKRD2 is associated with a favorable survival outcome, whereas the high expression of TNFRSF19 indicates an unfavorable survival ratio. E) Clinical patients with low expression of ANKRD2 and high expression of TNFRSF19 (n = 43) had a worst prognosis compared to F) those with high expression of ANKRD2 and low expression of TNFRSF19 (n = 43), G) high expression of ANKRD2 and TNFRSF19 (n = 17), H)and low expression of ANKRD2 and TNFRSF19 (n = 16).

Table 1
Clinical information of OSCC patients.

ANKRD2					TNFRSF19				
		+	–	P value			+	–	P value
Age	Mean ± SD	57.64 ± 11.22	57.68 ± 11.30	0.9849	Age	Mean ± SD	57.57 ± 11.29	57.60 ± 11.34	0.3703
Gender	Male	31(26.50 %)	36(30.70 %)		Gender	Male	36(30.77 %)	31(26.50 %)	
	Female	27(23.07 %)	23(19.66 %)	0.4124		Female	28(23.93 %)	22(18.80 %)	0.8093
T stage	1	7(5.98 %)	2(1.71 %)	0.0007	T stage	1	2(1.71 %)	7(5.98 %)	0.0285
	2	20(17.09 %)	6(5.13 %)			2	10(8.55 %)	16(13.68 %)	
	3	27(23.08 %)	45(38.46 %)			3	48(41.03 %)	24(20.51 %)	
	4	4(3.42 %)	6(5.13 %)			4	4(3.42 %)	6(5.13 %)	
Differentiation	high	23(19.66 %)	22(18.80 %)	0.3071	Differentiation	high	26(22.22 %)	19(19.24 %)	0.6552
	medium	22(18.80 %)	16(13.68 %)			medium	20(17.09 %)	18(15.38 %)	
	poor	13(11.11 %)	21(17.95 %)			poor	18(15.38 %)	16(13.68 %)	
Lymphatic metastasis	Yes	27(23.08 %)	47(40.17 %)	0.0002	Lymphatic metastasis	Yes	48(41.03 %)	26(22.22 %)	0.0035
	No	31(26.50 %)	12(10.26 %)			No	16(13.68 %)	27(23.08 %)	
Location	lingual margin	25(21.37 %)	21(17.95 %)		Location	lingual margin	22(18.80 %)	24(20.51 %)	
	dorsum linguae	8(6.84 %)	4(3.12 %)			dorsum linguae	6(5.13 %)	6(5.13 %)	
	gingiva	5(4.27 %)	8(6.84 %)			gingiva	8(6.84 %)	5(6.84 %)	
	floor of mouth	9(7.69 %)	7(5.98 %)			floor of mouth	8(6.84 %)	8(6.84 %)	
	buccal	7(5.98 %)	14(11.97 %)			buccal	14(11.97 %)	7(5.98 %)	
	palate	1(0.85 %)	2(1.71 %)			palate	2(1.71 %)	1(0.85 %)	
	lip	1(0.85 %)	1(0.85 %)			lip	2(1.71 %)	0(0 %)	
	oropharynx	2(1.71 %)	2(1.71 %)	0.2073		oropharynx	2(1.71 %)	2(1.71 %)	0.1623

prognosis of patients with a high expression of ANKRD2 was significantly better than that of patients with a low expression of ANKRD2 ($P = 0.0125$) (Fig. 3C). Patients with high TNFRSF19 expression showed a poor prognosis ($P = 0.0006$) (Fig. 3D). Patients with low ANKRD2 expression and high TNFRSF19 expression had the worst survival outcomes, which was consistent with the conclusions of our risk model (Fig. 3E). Patients with high expression of ANKRD2 combined with low expression of TNFRSF19 (Fig. 3F), high expression of ANKRD2 combined with low expression of TNFRSF19 (Fig. 3G), or low expression of ANKRD2 combined with low expression of TNFRSF19 (Fig. 3H) significantly better survival than patients with high expression of TNFRSF19 and low expression of ANKRD2 ($P = 0.0008$, $P = 0.0400$, and $P = 0.0211$, respectively). One-way analysis of variance (ANOVA) was performed to analyse the follow-up information of our clinical patients, and we found that TNFRSF19 and ANKRD2 were significantly correlated with the clinical stage ($P = 0.0007$ and $P = 0.0285$, respectively) and lymphatic metastasis ($P = 0.0002$ and $P = 0.0035$, respectively) (Table 1). The chi-square test demonstrated a significant correlation between ANKRD2 and TNFRSF19 expression levels ($P = 0.0001$) (Table 2), which again indicated that the combined effects of ANKRD2 and TNFRSF19 affect the prognosis of OSCC patients.

4. Discussion

OSCC is one of the most prevalent cancers in the world and has high morbidity and mortality [1]. The characteristics of OSCC, including in situ invasion, spread, lymph node metastasis, and distant metastasis, increase the difficulty of therapy [24]. Currently, surgery, chemotherapy, and radiotherapy are still the major strategies for OSCC treatment. As an important chemotherapy drug, cisplatin has been widely used in cancer treatment [25], but, increasing numbers of patients have emerged exhibiting chemoresistance [26], leading to a negative prognosis. Therefore, it is very important to predict the cisplatin sensitivity of OSCC patients so that the

Table 2
Chi-square test between TNFRSF19 and ANKRD2.

	ANKRD2+	ANKRD2-		P value
TNFRSF19+	21	43	64	0.0001
TNFRSF19-	37	16	53	
	58	59	117	

therapeutic strategy can be changed right away if need. The establishment of a reliable risk prediction model for chemotherapy represents a vital direction for precision medicine. Following the instructions of risk prediction models, scientists and physicians can infer mechanisms and biological functions [27]. The advantage of a nomogram is that it transforms complex regression equations into simple visual graphs, which makes the results of prediction models more readable and has a higher application value [28]. This advantage has led nomograms to receive increased attention and application in medical research and clinical practice. Herein, we built an ANKRD2-TNFRSF19 collaborative risk prediction model for OSCC patients.

TNFRSF19 (TROY), an orphan member of the tumour necrosis factor (TNF) superfamily, can competitively bind to TGFβ receptor type I (TβRI) in the cytoplasm, thereby blocking the binding between Smad2/3 and TβRI and resulting in the failure of Smad2/3/4 complex formation, the blockade of the TGFβ pathway, and the inhibition of cell proliferation becoming invalid in nasopharyngeal carcinoma [29]. High expression of TNFRSF19 can be detected in embryos, but it has only low expression in the colon, rectum, and small intestine in adulthood. The high expression of TNFRSF19 usually causes the malignant proliferation of cells, such as melanoma [30] and malignant glioma, which also predicts poor prognosis [31]. TNFRSF19 expression is correlated with nuclear factor kappa-B (NF-κB) expression, and the C-terminus of the isoform TNFRSF19.2 can trigger the NF-kB pathway, but its expression is also directly regulated by β-catenin/TCF4 binding to the TNFRSF19.2 promoter, thereby initiating the NF-kB pathway [32]. Epidermal growth factor receptor (EGFR) can combine to form a complex with TNFRSF19 and then activate the NF-κB pathway [33]. Lymphotoxin-α (LTα) promotes epithelial-mesenchymal transition (EMT) by combining with TNFRSF19 to form a complex that boosts skin epithelial development [34]. ANKRD2 is a direct target of miR-205-5p. LncRNA VENTXP1 regulates ANKRD2 through miR-205-5p, which reduces proliferation by downregulating NF-κB signalling. VENTXP1 inhibits the proliferation and clonal formation of head and neck squamous cell carcinoma cells [35]. Under oxidative stress, not only does VENTXP1 downregulate NF-κB via Akt phosphorylation but ANKRD2 also directly acts on p50, which is a subset of the NF-κB pathway, to inhibits the activation of the pathway [36]. Most importantly, both TNFRSF19 and ANKRD2 have complex neoplastic regulation mechanisms that have not been fully elucidate, especially in OSCC, which should be explored more in the future.

In our study, high expression of TNFRSF19 along with low expression of ANKRD2 indicates an unsatisfactory prognosis of OSCC patients. We also found that TNFRSF19 and ANKRD2 were significantly correlated with clinical stage and lymphatic metastasis. Moreover, the chi-square test demonstrated a significant correlation between ANKRD2 and TNFRSF19 expression, again indicating that the combined effect of ANKRD2 and TNFRSF19 contributed the poor prognosis of OSCC patients. This model may provide a direction to predict the outcome of cisplatin chemotherapy and guide clinical medicine to select different treatment regimens based on the individual circumstances of patients at an early stage that may improve survival in OSCC patients. However, the mechanism of TNFRSF19 and ANKRD2 in OSCC especially their influence on the prognosis of OSCC patients is still unclear and further exploration is necessary.

Nonetheless, the use of this model of ANKRD2 in combination with TNFRSF19 to assess the prognosis of patients with OSCC has the limitation that the model was only examined in patients with OSCC undergoing cisplatin chemotherapy following surgery. Multiple complex conditions, such as the dosage of cisplatin, other therapeutic combinations, and patient complications, are not included in our model considerations, which affects the versatility and accuracy of the model. The model flexibility needs to be improved in the future.

5. Conclusion

We developed a cisplatin sensitivity risk model by analyzing data from the TCGA database and clinical information from OSCC patients. In our risk model, expression of TNFRSF19 combined with expression of ANKRD2 predicted outcomes in OSCC patients undergoing surgical excision therapy and post-surgical chemotherapy. Although this model is in its infancy, there is still much room for improvement , this model may identify novel directions for studying the mechanisms of oral squamous cell carcinoma and provide new strategies for OSCC therapy.

Ethics approval and consent to participate

This research was conducted in accordance with international guidelines and the ethical standards outlined in the Declaration of Helsinki. This study was approved by the Second Affiliated Hospital of Shantou University Medical College Institutional Review Board. (Number: 2022-106; Registry Time: 23/08/2022).

Written informed consent were obtained from all the patients.

Consent for publication

Not applicable.

Data availability statement

Data included in article/supp. material/referenced in article.

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CRediT authorship contribution statement

Shucong Yao: Writing - review & editing, Writing - original draft, Formal analysis. **Hongwei Xiao:** Investigation, Data curation. **Changji Wei:** Methodology, Formal analysis. **Shisheng Chen:** Writing - review & editing, Project administration, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24091.

List of abbreviations

TCGA	The Cancer Genome Atlas database
OSCC	Oral squamous cell carcinoma
AJCC	American Joint Committee on Cancer
UICC	the Union for International Cancer Control
PBS	phosphate-buffered saline;
RT-PCR	Reverse Transcription-Polymerase Chain Reaction
TNF	tumor necrosis factor
TβRI	TGFβ receptor type I
NF-κB	nuclear factor kappa-B;
EGFR	Epidermal growth factor receptor
LTα	Lymphotoxin-α;
EMT	epithelial-mesenchymal transition

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