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Original Article

The expression of immune co-stimulators as a prognostic predictor of head and neck squamous cell carcinomas and oral squamous cell carcinomas

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KEYWORDS

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Abstract *Background/purpose:* T cells require second immune checkpoint molecules for activation and immune memory after antigen presentation. We found that inducible co-stimulator (ICOS) has been a favorable prognostic factor amongst B7 immune checkpoint co-stimulators (ICs) families in head and neck squamous cell carcinoma (HNSCC) and oral SCC (OSCC).

Materials and methods: This study analyzed the expression of non-B7 tumor necrosis factor (TNF) superfamily ICs in the Cancer Genome Atlas (TCGA) HNSCC cohort, our OSCC cohort, and TCGA pan-cancer datasets. The correlation in expression, prognosis, and immune status was assessed.

Results: The higher expression of CD27, CD30, CD40L, death domain 3 (DR3), and OX40, presumably on the T cell surface, defined better overall survival of HNSCC patients. Besides, CD27, CD30, CD40L, and OX40 were highly correlated with ICOS expression in tumors. CD27, CD40L, and DR3 expression are higher in HPV+ HNSCC tumors than in HPV- tumors. The combined expression level of CD27/OX40 or CD27/CD40L/OX40 enables the potent survival prediction of small, less nodal involvement, early stage, and HPV + tumor subsets. Tumors expressing

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high CD27, CD30, CD40L, ICOS, and OX40 exhibited enhanced immune cell infiltration. The high correlation in the expression of these ICSs was also noted in the vast majority of tumor types in TCGA datasets.

Conclusion: The findings of this study not only confirm the potential of the concordant stimulation of CD27, CD30, CD40L, ICOS, and OX40 as a crucial strategy in cancer immunotherapy but also inspire further exploration into the field, highlighting the promising future of cancer treatment.

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Introduction

Head and neck squamous cell carcinoma (HNSCC), including oral SCC (OSCC), is one of the leading causes of cancer death worldwide. Despite advances in therapies, the 5-year survival rate of HNSCC/OSCC patients remains around 50% over the past decades. The prognosis of HNSCC, especially advanced-stage tumors, remains poor because of the resistance to therapy, relapse, and distal metastasis. Therapeutic targeting of immune components in tumor microenvironment (TIME) is a modern therapeutic modality against malignancies.¹ Immune checkpoint blockade (ICB) drugs, especially those on PD-1, have achieved marked progress in tumor interception, including the therapies against HNSCC.^{2–5} However, delineating the immune checkpoint signature and the tumor-infiltrating lymphocytes (TILs) profile in TIME and defining their clinical implication would validate more effective tumor targeting to improve patient outcomes.^{6,7}

Following antigen presentation, T cells require second immune checkpoint molecules (ICMs), including immune co-stimulators (ICSs) or immune co-inhibitors for activation and immune memory. ICMs consist of B7 and non-B7 subgroups. Among 12 B7 ICM members, we have elucidated that inducible co-stimulator (ICOS) is the most crucial prognostic predictor in TCGA HNSCC dataset and our OSCC cohort.^{8,9} Non-B7 ICS family comprising at least seven paired tumor necrosis factor (TNF) family ligand/receptor complexes including 4-1BB (CD137), CD27, CD30, CD40L, death domain 3 (DR3), glucocorticoid-induced TNFR-related receptor (GITR) and OX40 (CD134) on the T-cell and their coupled 4-BBL, CD70, CD30L, CD40, TNF-family ligand (TL1A), GITRL and OX40L on the antigen-presenting cells (APCs), other immune cell types or tumor cell surface.¹⁰ ICSs have garnered significant attention recently for mechanistic approaches or being designed as co-targeting to facilitate tumor immunity.^{10,11}

Aberrant 4-1BB (CD137) expression has been found on TIL and peripheral blood lymphocyte (PBL) of HNSCC patients.^{11–13} CD27/CD70 (CD27 ligand) complex transduces signals leading to NFκB and JNK activation, which regulate B-cell activation and immunoglobulin synthesis.¹⁴ CD27/CD70 appeared to be the diagnostic markers or survival predictors of HNSCC,^{15,16} and CD27 agonists could be adjuvant to anti-PD1 therapy.¹⁷ Chimeric antigen receptor

(CAR)-T cell targeting CD70 has been developed to intercept HNSCC.¹⁸ Likewise, CD30 also contributes to the activation of NFκB and MAPK, which results in pro-survival effects.¹⁹ It is a marker of activated lymphocytes highly expressed in hematopoietic malignancies and is being tested as a CAR-T therapeutic target in refractory lymphomas.^{20,21} However, the expression profile of CD30/CD30L in HNSCC has been hitherto undefined. The gradual decrease of CD40 expression in monocytes and CD40L expression in T cells was noted during HNSCC progression.^{6,22} The antitumor effect on the CD40 expression was associated with the cytokine production. The signals of TL1A functioning through DR3 are a target of TNFα, which drives pluripotential activities in immunity and tumor pathogenesis.^{23–25} GITR is upregulated in TIL and T cell subsets of PBL in HNSCC patients relative to normal controls.¹³ The expression of OX40 is primarily expressed on activated T cells, and it mediates memory, anti-apoptosis, proliferation, and survival.^{26,27} OX40+ T cells are found in various solid malignancies and are prevalent in HNSCC.^{13,26,27} Due to the tumor suppressive activity, clinical trials of OX40-directed immune stimulation therapy against HNSCC are undergoing.^{5,26,28}

Although immune inhibitors have shown remarkable progress in cancer therapy,⁴ precision targeting is still needed to improve patient outcomes. This study, by providing a comprehensive insight into immune stimulators in the TIME of HNSCC, offers a potential avenue for more effective immunotherapy. The profiling of the ICS landscape identified that CD27, CD30, CD40L, DR3, ICOS, and OX40 were determinants of HNSCC patient survival. These ICSs could serve as clinical markers or promising co-targets for HNSCC therapy, instilling hope for improved patient outcomes.

Materials and methods

HNSCC transcriptional signatures

The patient information for HNSCC and normal tissue information was sourced from The Cancer Genome Atlas (TCGA, <https://portal.gdc.cancer.gov/>). This dataset, hereon defined as TCGA HNSCC cohort, provided a reliable basis for our cross-comparison, using the

expression matrix of genes based on the fragments per kilobase of transcript per million mapped reads (FPKM) format normalizing samples.

OSCC transcriptional signatures

RNA-Seq data from 54 OSCC patients and 28 matched normal tissues of our cohort were established previously.⁸ The cohort characteristics were described in our previous study. Transcripts per kilobase of transcript per million (TPM) mapped reads designated the clean reads of RNA-Seq.

Cell culture, induction of isografts, and RNA-Seq

The murine OSCC cell line MTCQ1, a key component of our study, was cultivated as previously described.²⁹ The inoculation of MTCQ1 isografts into the buccal space of C57BL/6J mice (National Laboratory Animal Center, Taipei, Taiwan) was performed.³⁰ This approach, along with the subsequent resection, processing, and RNA-Seq analysis of the grafts and the adjacent buccal mucosa, insighted the changes of gene transcription in murine syngeneic OSCC tumors. The expression matrix of genes shown as TPM values was then subjected to analysis. The animal study was approved by the Institutional Animal Care and Use Committee (IACUC) of National Yang Ming University (IACUC approval no.: 1080602).

Pan-cancer transcriptional signatures

The pan-cancer profile in the TCGA database, including 32 cancer studies, was achieved from the TIMER dataset (<https://cistrome.shinyapps.io/timer/>) or the ENCORI portal (<https://rnasyu.com/encori/>). Bubble plots and heatmaps designated the differences across settings.

Bioinformatic analysis

A comprehensive bioinformatic analysis, a cornerstone of our study, was conducted using EPIC (E), MCPCOUNT (M), QUANTISEQ (Q), TIMER (T), and XCELL (X) modes.³¹ This thorough approach allowed us to analyze immunization-related information, tumor-infiltrating immune cells, and variable cellular contexts. The resulting heatmaps illustrated the differential landscape across settings, further validating our findings.

Statistics

Data were presented as mean $\pm$ standard error (SE) or other formats. Mann-Whitney test, *t*-test, and correlation analysis were performed. Kaplan-Meier survival analysis was used to assess the overall survival (OS). The prediction accuracy of the patient survival was evaluated by time-dependent receiver operating characteristic curve (ROC) mode. The prognostic signatures were also analyzed using a

multivariate Cox regression module and illustrated using Lollipop plots.

Results

Dysregulation of immune co-stimulators in HNSCC and OSCC

Analysis of 7 paired non-B7/CD28 ICS ligand-receptor complex molecules, including 4-1BB/4-1BBL, CD27/CD70, CD30/CD30L, CD40L/CD40, DR3/TL1A, GITR/GITRL, and OX40/OX40L was carried out in 44 normal tissue and 502 HNSCC tumors from the TCGA HNSCC cohort. It revealed the upregulation of 4-1BB, CD27, CD30, DR3, GITR, and OX40 expression and the downregulation of CD40L expression, presumably expressed in the immune cell fraction of tumor tissues compared to normal counterparts. In addition, the coupled 4-1BBL, CD70, CD40, and OX40L, presumably expressed in tumor cell or APC fraction, was also upregulated in tumors (Fig. 1A). The co-upregulation of 4-1BB/4-1BBL, downregulation of CD40L/upregulation of CD40, upregulation of CD70 and OX40L, and the downregulation of TL1A being identified in our OSCC cohort was in agreement with findings in TCGA HNSCC cohort (Fig. 1B). Among 89 HNSCC examined with in situ hybridization, 22 (24.7%) are HPV positive. The HPV+ tumors exhibit a better survival relative to HPV- cases (not shown). Of note, the expression of CD27, CD40L, and DR3 was higher in HPV+ HNSCC in relation to HPV- HNSCC (Fig. 1C). Except for CD40L, the expression of most ICSs was also higher in the murine isograftic MTCQ1 tumors than control oral mucosa (Fig. 1D). The general upregulation of ICSs was seen in TCGA HNSCC tumors, human OSCC and murine OSCC isografts.

Association of higher CD27, CD30, CD40L, DR3, ICOS, and OX40 expression with better HNSCC survival

The higher expression of CD27, CD30, CD40L, DR3, OX40, and ICOS, presumably localized on the T cell surface, defined the better overall survival of HNSCC tumors in Kaplan-Meier plots, with hazard ratios 0.61, 0.40, 0.50, 0.68, 0.63 and 0.67, respectively (Fig. 2A and B). The expression of other non-B7 ICSs was not associated with HNSCC survival. The expression of ICSs was also the prognostic predictor of other malignancies in the TCGA dataset, either for favorable or unfavorable prognosis. Nevertheless, the prediction power of these ICSs in HNSCC stands on the leading rank in the malignancies exhibiting favorable prognosis (Fig. 2C). Multivariate logistic regression specified the expression level of each molecule as an independent prognostic predictor of HNSCC (Fig. 2B). CD27 was the best solitary independent prognostic predictor of HNSCC. Besides, the higher ICOS expression in HPV+ HNSCC defined a worse prognosis (Fig. 2D). Since there was remarkable inter-correlation across the expression of CD27, CD30, CD40L, ICOS, and OX40 across HNSCC and OSCC. However, no consistent correlation between DR3 and other ICSs was

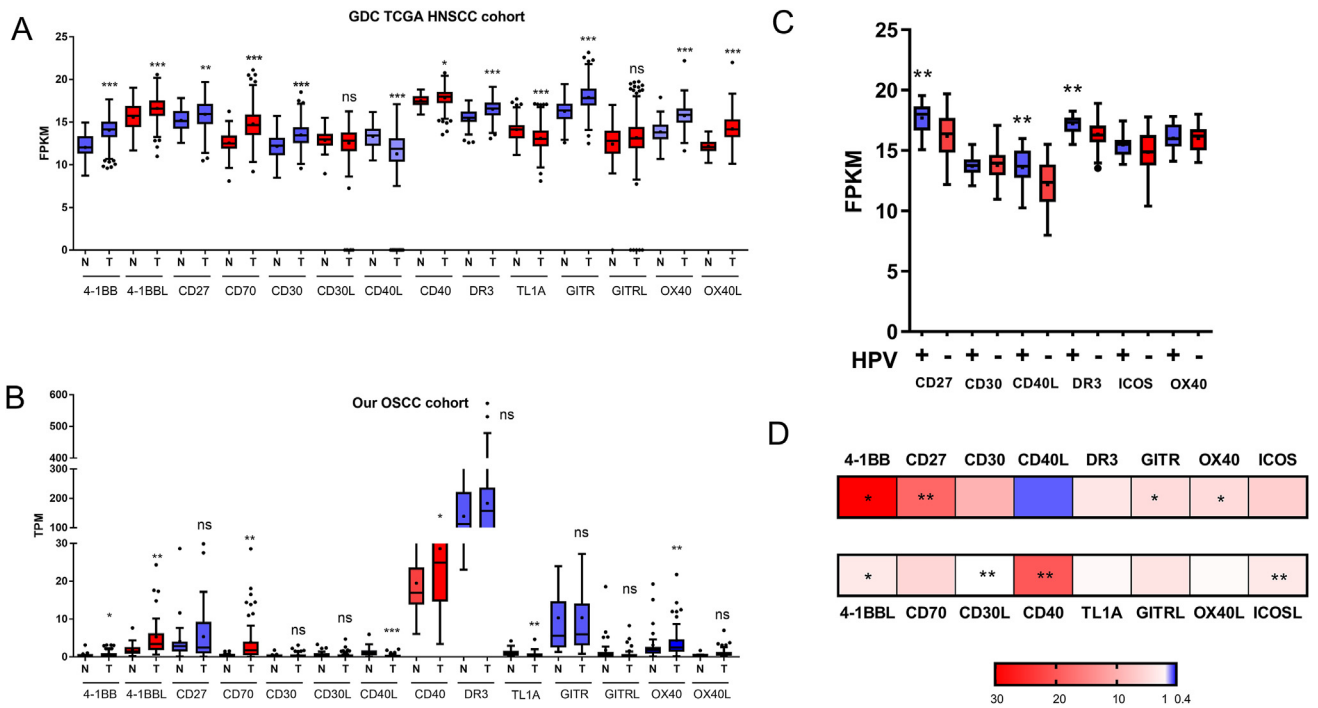


Figure 1 Transcriptomic profiles of immune co-stimulators. (A–C) Box and Whiskers plots. (A) TCGA HNSCC tumors. (B) Our OSCC cohort. (C) TCGA HNSCC tumors are characterized to be HPV+ or HPV-. N is normal tissue; T is tumor tissue. +, medium value. Y-axis in HNSCC, FPKM; Y-axis in OSCC, TPM. (D) Heatmap to illustrate the ratio of mean TPM in 6 individual MTCQ1 buccal isografts/mean TPM in 3 individual buccal mucosa tissues. HNSCC, head and neck squamous cell carcinoma; OSCC, oral squamous cell carcinoma. *ns*, not significant. *, ** and *** represent $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. *t*-test or Mann-Whitney test.

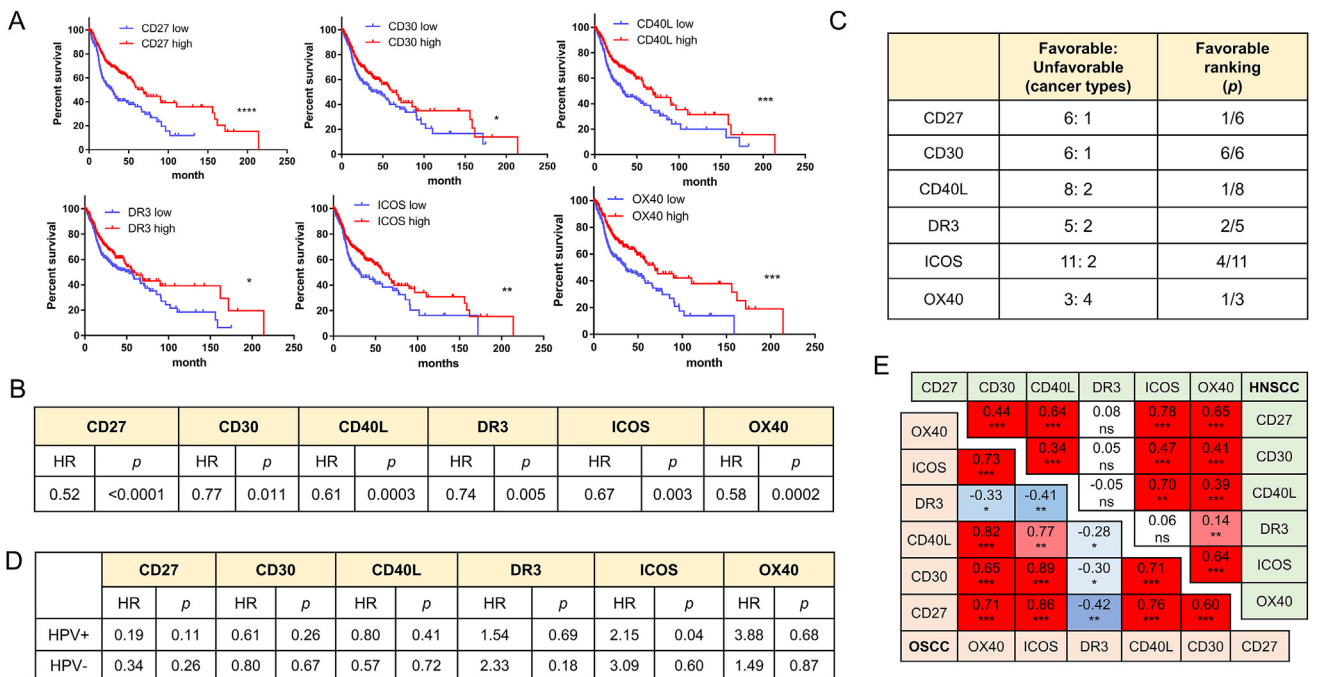


Figure 2 The implications of CD27, CD30, CD40L, DR3, ICOS and OX40 expression. (A–C) Survival analysis. (A) Kaplan-Meier curve analysis of immune co-stimulators (ICs) in HNSCC tumors. (B) The hazard ratio and *P* value are related to each ICs in (A). (C) The states of these ICs in malignancies. Lt column shows the states of high expression of these ICs as favorable or unfavorable prognostic markers. Numbers, the number of cancer types. The Rt column shows the rank of *P* values of these ICs in HNSCC as related to those in all favorable tumors. (D) The survival analysis of these ICs according to HPV states. (E) Heatmap illustrating the inter-correlation in the expression of these ICs in HNSCC and OSCC. HNSCC, head and neck squamous cell carcinoma; OSCC, oral squamous cell carcinoma. Numbers, *r* value; stars, *P* values. *ns*, not significant. *, **, *** and **** represent $P < 0.05$, $P < 0.01$, $P < 0.001$, $P < 0.0001$, respectively. Correlation analysis.

found (Fig. 2E), and DR3 was excluded in the subsequent analysis.

Combined analysis of immune co-stimulators for prognostic prediction of HNSCC subsets

Multivariate logistic regression specified the combined expression level of ICs, except for one combination, as independent prognostic predictors of overall survival in HNSCC (Fig. 3A, Table S1). Moreover, the ROC analysis of one-year to three-year follow-up confirmed that the discrimination power of the combined ICs analysis was time-dependent (Fig. 3B). Moreover, the combination of OX40 or CD40L/OX40 increased the prediction power of CD27 in the survival of T1,2, N0, 1 and HPV+ tumor subsets (Fig. 3C). Representative analyses are shown in Fig. 3D. The CD27, CD40L, and ICOS expressions were associated with differentiation grade in both HNSCC and OSCC cohorts. However, the association trend appeared diverse between them (Fig. S1).

Homogenous expression of immune co-stimulators within major human malignant tumors

The diverse landscape of CD27, CD30, CD40L, ICOS, and OX40 expression between major cancers, including breast

invasive carcinoma (BRCA), colorectal adenocarcinoma (COAD), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), and glioblastoma (GBM), and their paired normal tissues in the TCGA database was notified (Fig. 4A). Except for COAD, CD27 expression in tumors was high in tumors. Unlike HNSCC, CD30 was downregulated in most major malignancies. Like HNSCC, CD40L was downregulated, and OX40 was upregulated in most malignancies. The expression states of CD40L, ICOS, and OX40 were relatively homogeneous in major malignancies (Fig. 4B). The expression of ICs in GBM was generally lower than in other tumors.

Immerse inter-correlation across CD27, CD30, CD40L, ICOS, and OX40 expression in pan-cancers

Despite the variation in expression, the inter-correlation of each two of the dysregulated ICs was extremely high amongst tumors as in 88% (282/320) of comparison pairs; the correlation was highly significant (Fig. 5A, Table S2–Table S11). In 53% (17/32) types of malignancies, the expression of all these five molecules was highly correlated. Fig. 5B shows the r values in major malignancies. The correlation of ICs in BRCA and LUSC was extremely high. The high correlation between CD27 and ICOS and between CD40L and ICOS was noticed in all types of malignancies (Fig. 5A and B). However, such inter-correlation in immune

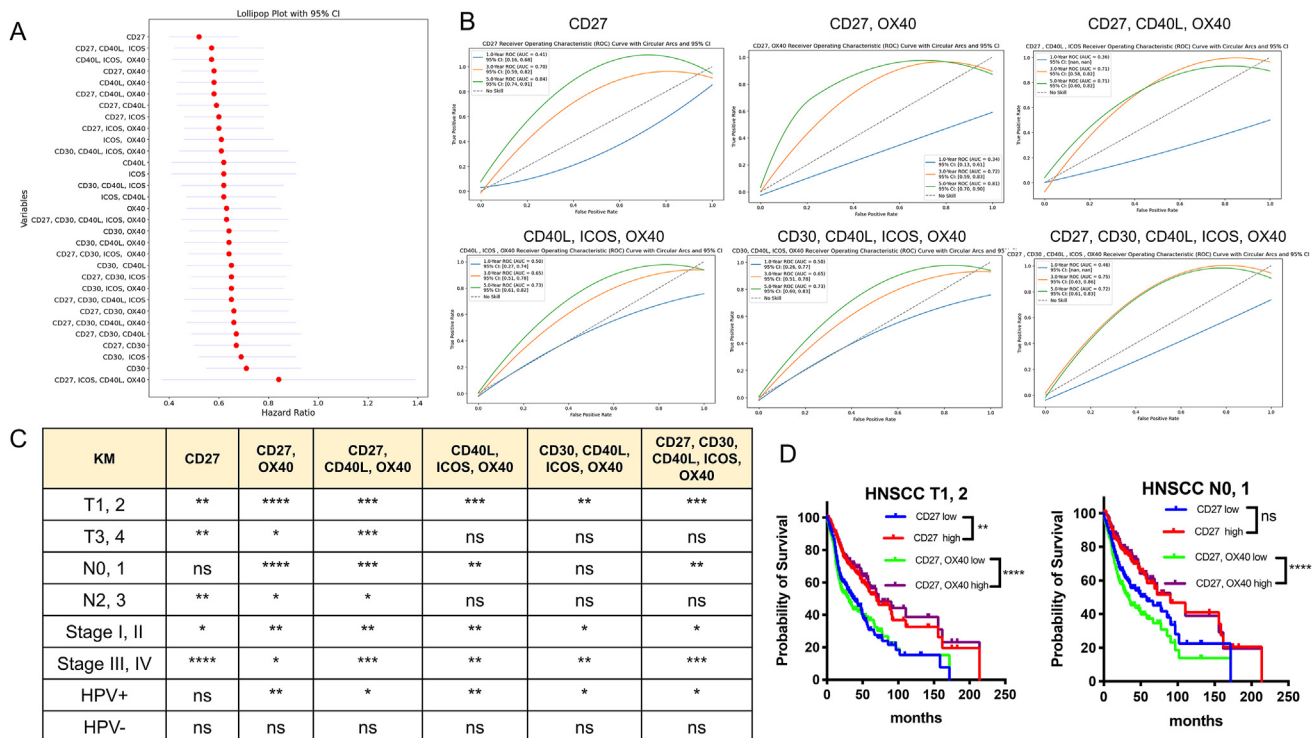


Figure 3 The prognostic implications of the combined analysis of CD27, CD30, CD40L, ICOS, and OX40 expression. (A) Lollipop plots to illustrate the hazard ratios (dots) and 95% confidence interval of 28 combined groups derived from a multivariate module. The risk scores are achieved from each combination's regression coefficients and expression level. The representative groups are subjected to analysis in (B, C). (B) Year-dependent ROC analysis of OS. Blue curve, one year; red curve, three-year; green curve, five-year. A year-dependent increase of AUC is notified in most analyzed groups. (C, D) Kaplan-Meier survival analysis. (C) Analysis of tumor subgroups using the medium values of risk scores of patients. (D) Representative analysis to compare the dissection power of CD27 vs CD27/OX40 in T1, 2 patient subgroup (Lt panel) and N0, 1 patient subgroup (Rt panel). HNSCC, head and neck squamous cell carcinoma. ns, not significant. *, **, *** and **** represent $P < 0.05$, $P < 0.01$, $P < 0.001$, $P < 0.0001$, respectively.

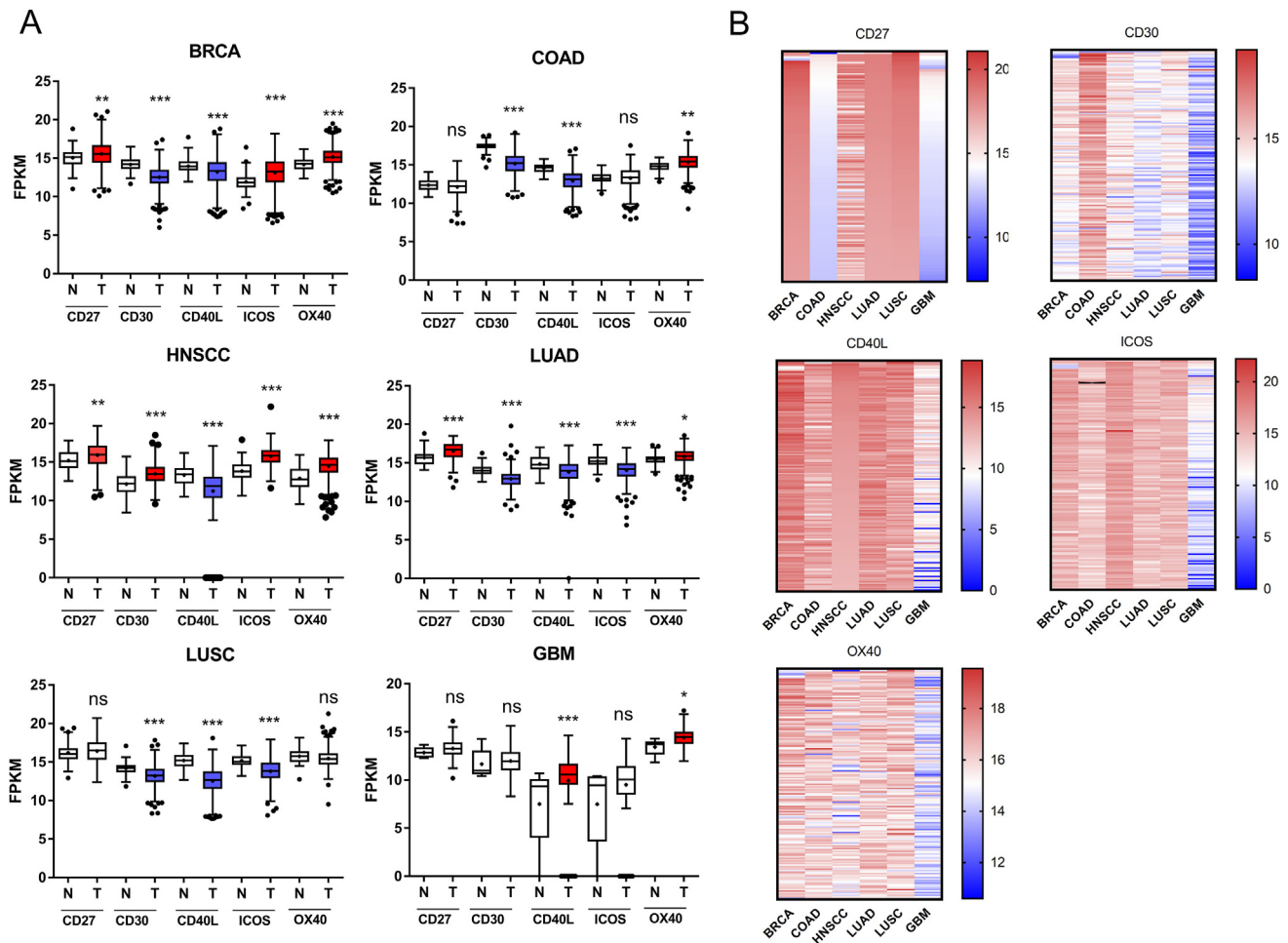


Figure 4 The expression of immune co-stimulators in major malignancies of breast, colon, lung, and brain in addition to HNSCC in TCGA dataset. (A) Box and Whisker's plots. The expression of ICSs in normal and tumor tissues in six TCGA cancer datasets. Y axis, FPKM; N, normal tissue; T, tumor tissue. +, medium value; Red box, upregulation; blue box, downregulation; white box, no significant change in expression. BRCA, breast invasive carcinoma; COAD, colorectal adenocarcinoma; HNSCC, head and neck squamous cell carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; GBM, glioblastoma. *ns*, not significant. *, **, and *** represent $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. *t*-test. (B) Heatmaps illustrating the FPKM of ICSs for individual tumors. Gradient bars delineate the FPKM for each ICS.

cell malignancies, including diffuse large B cell lymphoma (DLBC) and acute myeloid leukemia (LAML), was much weaker than non-immune malignancies.

Association between immune profile and the expression of immune co-stimulators in tumors

Bioinformatic algorithms revealed a substantially positive correlation between ICS expression level and immune scores, especially lymphocyte infiltration, including B cells, CD8⁺ T cells, macrophage, MDSC, and neutrophil in both HNSCC and OSCC tumor cohorts (Fig. 6A). High CD27, CD40L, and ICOS expression were particularly highly associated with TIL scores (Fig. 6A and B). The association between these molecules with stromal or endothelial cells was inconsistent across cohorts. These ICS molecules seemed negatively associated with other cells, mostly uncharacterized or tumor cells.

Discussion

HNSCC/OSCC exhibits multifaceted abnormalities and poses significant therapeutic challenges, with only a limited number of drugs demonstrating efficacy against this disease. Multiple immunotherapy strategies, including ICB drugs, co-stimulatory agonists, antigenic vaccines, adoptive T cell transfer, oncolytic virus therapy, and others, have been proposed to intercept malignancies.⁵ The profiling of ICM and TIL cell subsets in tumors would render the selection of appropriate targeting strategies. This study identifies the concordant upregulation of six of seven non-B7 ICSs in the TIL of HNSCC, albeit CD40L is downregulated. Besides, the upregulation of 4-1BB, CD27, and OX4 in TIL co-exists with the upregulation of matched 4-1BBL, CD70, and OX40L on tumor cells. Previous reports have only signified the co-upregulation of CD27/CD70 in HNSCC.^{15,16} The co-upregulation of 4-1BB/4-1BBL and OX40/OX40L,

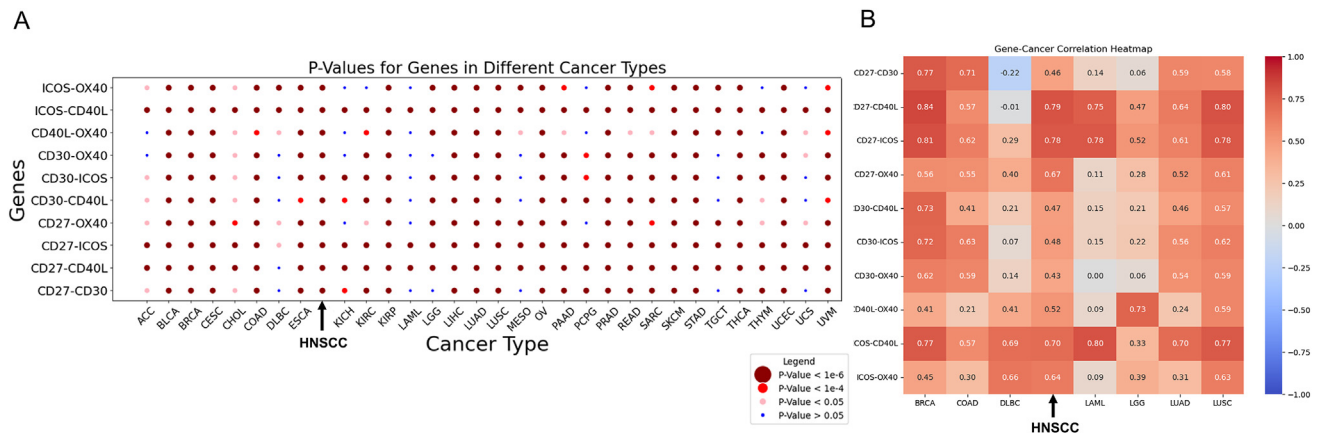


Figure 5 The pair-wise correlation analysis across ICSs in the TCGA pan-cancer dataset. (A) bubble plot illustrating the significance level of P values in pairs across ICSs (Y-axis) and 32 tumor types (X-axis). (B) Heatmap illustrating the r values in pairs across ICSs (Y-axis) and major tumor types (X-axis). The correlation profiles of DLBC and LAML are deviated from other tumors. ACC, Adrenocortical carcinoma; BLCA, Bladder urothelial carcinoma; BRCA, Breast invasive carcinoma; CESC, Cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, Cholangiocarcinoma; COAD, Colon adenocarcinoma; DLBC, Lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, Esophageal carcinoma; HNSCC, Head and neck squamous cell carcinoma; KICH, Kidney chromophobe; KIRC, Kidney renal clear cell carcinoma; KIRP, Kidney renal papillary cell carcinoma; LAML, Acute myeloid leukemia; LGG, Brain lower grade glioma; LIHC, Liver hepatocellular carcinoma; LUAD, Lung adenocarcinoma; LUSC, Lung squamous cell carcinoma; MESO, Mesothelioma; OV, Ovarian serous cystadenocarcinoma; PAAD, Pancreatic adenocarcinoma; PCPG, Pheochromocytoma and paraganglioma; PRAD, Prostate adenocarcinoma; READ, Rectum adenocarcinoma; SARC, Sarcoma; SKCM, Skin cutaneous melanoma; STAD, Stomach adenocarcinoma; TGCT, Testicular germ cell tumors; THCA, Thyroid carcinoma; THYM, Thymoma; UCEC, Uterine corpus endometrial carcinoma; UCS, Uterine carcinosarcoma; UVM, Uveal melanoma. Arrows indicate HNSCC.

which were newly found in this study, may denote their roles in tumor immune activation. Although the analytical power of our OSCC cohort is lower than that of the TCGA HNSCC sample cohort, probably owing to the sample size limitation, the expression profile of ICSs in OSCC is similar to that in HNSCC. Likewise, although the analytical strength of orthotopic syngeneic murine tumors is impeded by analyzing multiple grafts derived from the same cell line, the trend of ICM change in the mouse OSCC model simulates that of human HNSCC.²⁹ The findings confirm the eminent and concordant dysregulation of non-B7 ICS members in HNSCC. As eminent metastasis can be perceived in this model, the association between immune disruption and metastatic progression could be addressed using this model.^{29,32}

We found that the higher expression of CD27, CD30, CD40L, DR3, ICOS, and OX40 is associated with better patient survival in HNSCC. The high expression of these ICSs in tumors predisposes to a favorable rather than unfavorable prognosis. CD27 exhibits the most potent prediction power in HNSCC, next by OX40, CD40L, and ICOS. As the prediction power of CD27, CD40L, ICOS, and OX40 in HNSCC ranks in the leading edge among all malignancies, together with their high intercorrelation in expression, these ICSs could be considered HNSCC-specific prognosticators. Studies have revealed elevated expression levels of CD27 in HNSCC tumors compared to normal tissues, suggesting its pathogenetic implications. As higher expression of CD27 in HNSCC tumors has been associated with improved survival outcomes, the administration of CD27 agonists has been suggested by previous research as a feasible strategy for HNSCC interception.¹⁷ This approach also suggests that strategies

aiming at modulating CD27 signaling may be explored as part of immunotherapeutic interventions once the findings are ascertained by protein-based analysis. The role of CD70 as a partner of CD27 also requires specification.⁷ ICOS has been an active immune response and has led to better patient survival in HNSCC/OSCC tumors.⁸ Another known effect is the prevalence of OX40⁺ T cells in HNSCC,^{13,26,27} which leads to potential OX40-directed immune stimulation therapy against HNSCC.^{26,28} CD40L is one of the favorable prognostic predictors of HNSCC.⁶ Our stratification highlights the enrichment of prognostic values in small, early-stage, less metastatic, and HPV + HNSCC subsets by combining these predictors with CD27. This comprehensive study provides additional clues substantiating that the co-upregulation of CD27, CD40L, ICOS, and OX40 could be essential targets in HNSCC immunotherapy. Although the diverse expression landscape across tumors underscores the need for tailored therapeutic approaches, simultaneous targeting of these costimulatory pathways may enhance the efficacy of immunotherapeutic interventions.

Since the concordance in the expression of CD27, CD30, CD40L, ICOS, and OX40 are eminent in HNSCC, we extended the expression analysis to a broad spectrum of malignancies. Although the expression levels of ICSs are divergent across major malignancies of the breast, colon, and lung when compared to their normal controls, the expression profiles of CD27, CD40L, and ICOS within the same type of tumor are generously homogenous, which may suggest the co-activation of these ICSs in these pivotal human cancers. The diverse expression in some types of normal tissue due to various degrees of inflammatory cellular infiltration or the paucity of samples may also complicate

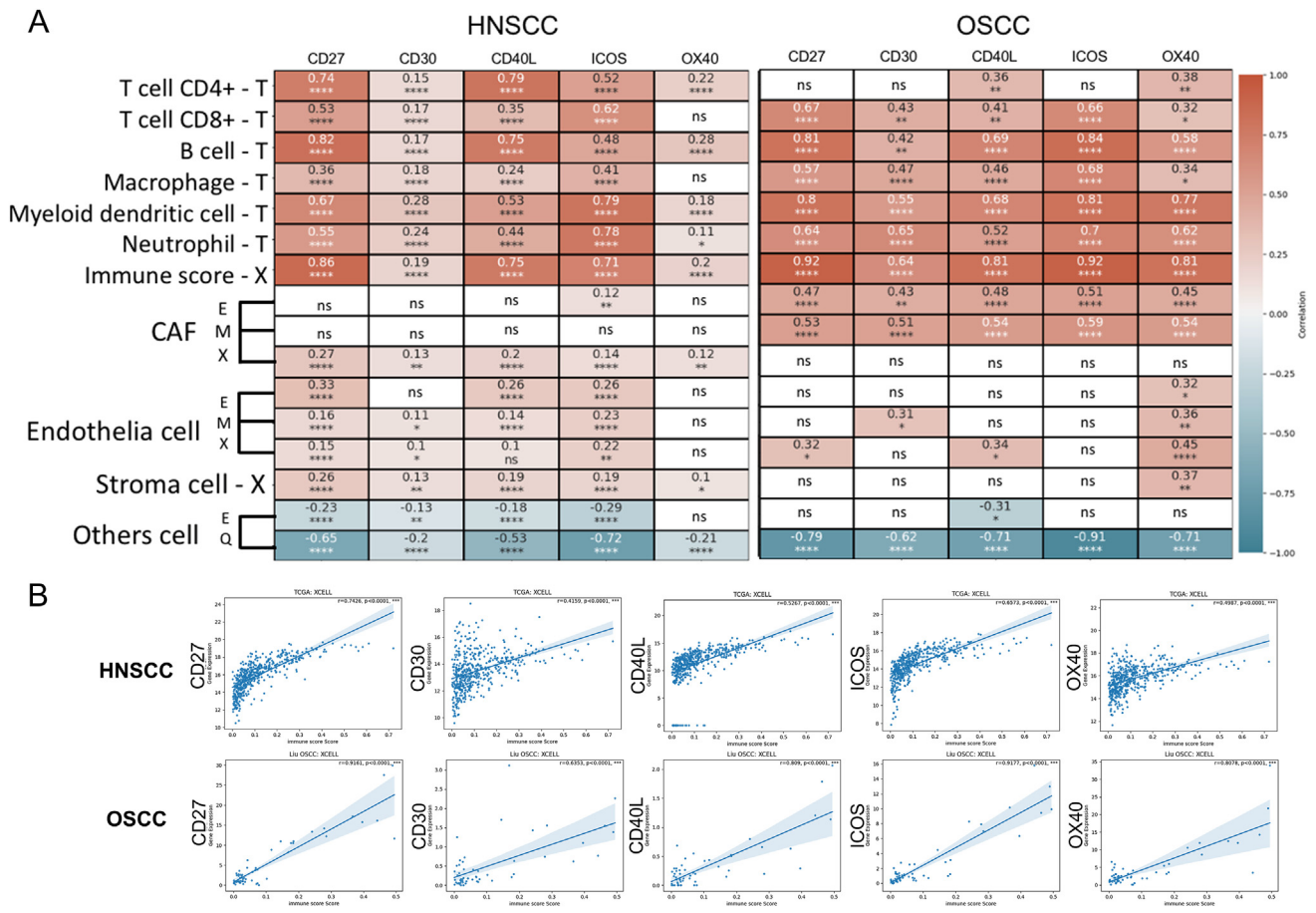


Figure 6 The immune cell profiles in HNSCC/OSCC. (A) Heatmaps illustrate the correlation between the scores of immune cells and ICSS. Lt, HNSCC. Rt, OSCC. Y-axis, cell type, Abbreviations, the analytical algorithms, Number and gradient bar, r value; star sign, P -value; E, EPIC, M, MCPCOUNT; Q, QUANTISEQ; T, TIMER; X, XCELL. CAF, cancer-associated fibroblast. HNSCC, head and neck squamous cell carcinoma; OSCC, oral squamous cell carcinoma. *, ** and **** represent $P < 0.05$, $P < 0.01$ and $P < 0.0001$, respectively. Other cells are most likely tumor cells. (B) Individual correlation analysis. Upper, HNSCC; Lower, OSCC; Y-axis, immune co-stimulators; X-axis, immune score revealed by XCELL program; line, correlation line; blue zone, 95% confidence interval.

the differences in expression across normal and tumor tissues. Alongside HNSCC, CD40L downregulation and OX40 upregulation are common in these major malignancies. Pan-cancer analysis further reveals the high inter-correlation of ICSSs in most malignancies. The high inter-correlation amongst CD27/CD40L/ICOS, especially the correlation between CD40L and ICOS, existing in nearly all tumors, may suggest the co-targeting values in global cancer therapy. The discrepancies between immune and non-immune solid malignancies highlight the unique characteristics of immune-related interactions in TIME. The findings in pan-cancer also suggest the existence of potential synergistic interaction or shared regulatory mechanisms, underscoring the complexity of immune checkpoint networks in cancers.⁵

The tumor immune score correlates with ICS expression and is compatible with favorable survival found in HNSCC patients with high ICS expression. The observed correlation with TIL further underscores the pivotal role of ICSSs in the TIME. Recent advances in ICB have provided valuable insights into managing complex cases. At the same time, the combined co-stimulation of ICSSs for TIL enrichment could be a potential adjuvant strategy.^{4,5} The comprehensive

analysis of the ICS profile would contribute significant insights for developing targeted therapies and personalized treatment strategies for HNSCC. This study delineates the prognostic significance of CD27, CD30, CD40L, ICOS, and OX40 expression in HNSCC, with CD27 demonstrating potent predictive power. Although confirming the findings in larger sample cohorts using more immune cell markers and the functional validation in experimental models remains to be specified, the combined assessment of these molecules provides valuable insights into risk stratification and prognosis for HNSCC patients, thereby advancing our understanding of cancer biology and immunotherapy.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jds.2024.05.004>.

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