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Integrating single-cell and spatial transcriptomics with pan-cancer bulk sequencing identifies OSR2 as a multifaceted biomarker for prognosis and tumor immunity

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Abstract

Background OSR2 plays a key role in various physiological processes and cancer development. Although interest in OSR2's role in cancer is increasing, its mechanisms and clinical importance are not fully understood. This study thoroughly examines OSR2 gene expression in different cancers and evaluates its potential as a prognostic marker and therapeutic target.

Methods A comprehensive pan-cancer analysis utilized single-cell RNA sequencing data from the CIDE database to examine OSR2 expression in various immune cells. Spatial transcriptomics from the CROST database explored OSR2's spatial expression in renal carcinoma tissues. RNA sequencing data from the TCGA database assessed OSR2's differential expression in 38 cancer types. The clinical significance of OSR2 was evaluated through its correlation with patient survival outcomes. Additionally, its impact on the tumor immune microenvironment was studied by analyzing associations with immunomodulatory factors, immune checkpoint molecules, and immune cell abundance.

Results The pan-cancer analysis revealed that OSR2 expression is often altered in various cancers. It is upregulated in 13 types, notably in gliomas like glioblastoma multiforme and low-grade glioma, and downregulated in 19 types, including kidney and bladder cancers. High OSR2 levels are linked to poor prognosis in several cancers and correlate with clinical factors such as age, gender, and tumor stage. Immunologically, OSR2 expression is positively associated with immunomodulatory and checkpoint genes in cancers like gliomas and colon cancer, but shows an antagonistic relationship with immune cell infiltration in prostate and pancreatic cancers.

Conclusion The findings suggest that OSR2 expression varies across cancer types and is linked to patient prognosis and changes in the tumor immune environment, making it a promising therapeutic target. Its role in tumor progression and immune interactions indicates its potential as a biomarker for cancer immunotherapy. Further

research is needed to understand OSR2's molecular mechanisms in cancer and to validate its use in immunotherapy.

1 Introduction

The pathogenesis of cancer is intricately associated with a variety of genetic mutations and encompasses numerous distinct subtypes and genetic backgrounds [1, 2]. Cancer progression is driven by oncogene overexpression and tumor-suppressor inactivation, arising from either lifetime-acquired somatic mutations or germline alterations [3, 4].With advances in genomic, epigenomic, and transcriptomic analyses, multi-omics approaches are now widely applied across cancers to enable tumor stratification, improve prognostic prediction, guide treatment response, and reveal actionable therapeutic targets [5–7].Despite the critical importance of tumor heterogeneity in oncological research, its assessment through specific biomarkers and the translation of this understanding into clinical interventions remain substantial challenges. Concurrently, advances in integrated cancer management—including targeted therapy and adjunctive approaches such as traditional Chinese medicine—have opened additional avenues for improving treatment outcomes [8–10].

OSR2 encodes a zinc-finger transcription factor that is subject to epigenetic regulation during cellular quiescence, influencing genes associated with proliferation and development [11, 12].Research has elucidated the role of OSR2 in skeletal growth and morphogenesis through its regulation of multiple signaling pathways, including the BMP and Sema3 pathways [13], In zebrafish, OSR2 serves as a novel marker for proximal tubules, with its expression being useful for tracing the development of proximal tubules [14]. In mice, OSR2 is also expressed during kidney development [15]. Increasing evidence suggests that OSR2 plays a critical role in tumorigenesis, progression, therapy, and prognosis. Research has demonstrated that OSR2 exhibits a hypermethylated state in gastric cancer tissues compared to adjacent normal tissues [16];Comprehensive molecular characterization of the 8q22.2 region in bladder cancer(BLCA) has revealed that OSR2 mRNA expression holds prognostic significance in muscle-invasive BLCA [17]; Recent in-depth studies have elucidated that Osr2 integrates biomechanical signals by coupling T-cell receptor (TCR) signaling with biomechanical stress through the Piezo1/Ca^{2+}/CREB axis, thereby promoting the terminal exhaustion of tumor-reactive $CD8^{+}$ T cells [18].

Although OSR2 has been reported to be associated with various malignancies, its expression pattern across pan-cancer and its systematic relationship with the tumor immune microenvironment and prognostic characteristics remain to be elucidated. Therefore, this study conducts a systematic analysis of the pan-cancer expression characteristics of OSR2 and its potential clinical relevance based on public multi-omics resources.

2 Materials and methods

2.1 Single-cell sequencing and spatial transcriptomic analysis

Following previous studies [19–21], Publicly available single-cell RNA sequencing (scRNA-seq) data were accessed through the Cancer Immunotherapy Data Explorer (CIDE). The Lineage module was used to examine OSR2 expression based on the

platform's integrated single-cell reference datasets and default preprocessing and normalization pipelines. Spatial transcriptomic data were obtained from the Comprehensive Repository of Spatial Transcriptomics (CROST) database (dataset ID: VISDS000159). In this study, OSR2 spatial expression patterns, cluster assignments, and cell-type annotations were directly extracted from the CROST dataset and visualized using the database's built-in analytical and visualization platform.

2.2 Analysis of OSR2 fundamental background (HPA database/GeneMANIA website)

A comprehensive analysis of OSR2 was performed using the Human Protein Atlas and GeneMANIA databases, focusing on its protein structure, interacting genes, and subcellular localization via immunofluorescence.

2.3 Data acquisition, differential expression, and pan-cancer survival analysis with clinical phenotype correlation

In alignment with a prior study [22], we acquired a uniformly processed pan-cancer dataset—TCGA TARGET GTEx (PANCAN, N = 19,131, G = 60,499)—from the UCSC Xena database. We extracted OSR2 gene expression data, retaining samples from specific sources while excluding entries with zero expression. Expression values underwent log2(x + 0.001) transformation, and cancer types with fewer than three samples per category were omitted. The differential expression of OSR2 between tumor and normal tissues was evaluated using the unpaired Wilcoxon rank-sum test. Prognostic data, including OS, DSS, disease-free interval (DFI), and progression-free interval (PFI), for 38 cancer types were integrated from TCGA prognostic studies published in cell and the UCSC Cancer Browser. The prognostic significance of OSR2 expression was assessed utilizing Cox proportional hazards regression models, supplemented by Log-rank tests. Statistical associations with clinical characteristics, such as stage, sex, and age, were analyzed using t-tests and analysis of variance (ANOVA), respectively.

2.4 Analysis of OSR2 heterogeneity and stemness index in human tumors

Using previously outlined methods [20], microsatellite instability (MSI) scores for each tumor sample were obtained from the TCGA Pan-Cancer Atlas study "Landscape of Microsatellite Instability Across 39 Cancer Types" [23]. Neoantigen (NEO) burden data were sourced from "The Immune Landscape of Cancer" [24]. Spearman correlation analysis was used to examine the relationships between OSR2 expression and factors like tumor mutation burden (TMB), MSI, NEO, and tumor purity. DNA methylation-based stemness scores (DNAss) and mRNA-based stemness scores (RNAss) were acquired from "Machine Learning Identifies Stemness Features Associated with Oncogenic Dedifferentiation" [25]. Samples with zero expression values were excluded, and remaining data were normalized using a log2(x + 0.001) transformation. Cancer types with fewer than three samples per group were excluded from the stemness score analysis for statistical reliability.

2.5 OSR2 mutation landscape and gene mutation profiles across different cancer types

Level 4 Simple Nucleotide Variation datasets for all TCGA samples, processed using the MuTect2 software [26], were acquired from the Genomic Data Commons (GDC) portal (https://portal.gdc.cancer.gov/). The mutational landscape was analyzed utilizing the R

package maftools (version 2.2.10) to aggregate sample-level mutations and annotate protein domains. Patients were stratified into high and low OSR2 expression cohorts based on the median expression value. Subsequent analyses concentrated on investigating the ten most frequently mutated genes across eight selected cancer types (BLCA、CESC 、DLBC、HNSC、KICH、KIRP、LGG、MESO) within the framework of this OSR2 expression stratification.

2.6 Analysis of the correlation between OSR2 expression and immune modulator genes, immune checkpoint genes, and immune cells

The uniformly standardized pan-cancer dataset, TCGA TARGET GTEx (PANCAN, N = 19,131, G = 60,499), was obtained from the UCSC Xena database (Link 1). Gene expression data were extracted for OSR2 (ENSG00000164920) and a panel of 150 genes representing five immune pathway categories: chemokines (41 genes), receptors (18 genes), MHC molecules (21 genes), immunoinhibitors (24 genes), and immunostimulators (46 genes). For all samples, data pertaining to OSR2 (ENSG00000164920) and 60 immune checkpoint marker genes were further obtained. These checkpoint genes were categorized into Inhibitory (24 genes) and Stimulatory (36 genes) groups, following the classification outlined in “The Immune Landscape of Cancer" [24].To further evaluate immune cell infiltration, we utilized the deconvo_epic method (EPIC: Simultaneous enumeration of cancer and immune cell types from bulk tumor gene expression data [27]) available in the R package IOBR (version 0.99.9, Link 2). The recalibration methodology was employed on the gene expression dataset to enhance the infiltration scores of key immune and stromal cell types, including B cells, T cell subsets ($CD4^+$, $CD8^+$), macrophages, natural killer (NK) cells, cancer-associated fibroblasts (CAFs), and endothelial cells, within each sample. This approach facilitated a comprehensive profiling of the correlation between OSR2 and the tumor immune microenvironment.

2.7 Analysis of OSR2 expression in relation to RNA modification-related genes, signaling pathways, and drug sensitivity

The uniformly standardized pan-cancer dataset, TCGA TARGET GTEx (PANCAN, N = 19,131, G = 60,499), was procured from the UCSC Xena database. From this dataset, expression data for the gene ENSG00000164920 (OSR2) and 44 marker genes associated with three categories of RNA modifications—m1A (10 genes), m5C (13 genes), and m6A (21 genes)—were extracted for all samples. We utilized a dual-faceted methodology via the GSCALite platform (http://bioinfo.life.hust.edu.cn/web/GSCALite/). Initial ly, we conducted a systematic evaluation of the correlation between OSR2 expression and the activity of cancer-associated signaling pathways. Subsequently, by integrating pharmacogenomic data from the GDSC and CTRP databases within GSCALite [28], we examined the relationship between OSR2 expression levels and sensitivity to anticancer drugs.

3 Results

3.1 Single-cell analysis results

scRNA-seq showed high OSR2 gene expression in stromal cell populations, with the highest levels observed in CAFs. Spatial transcriptomics of renal carcinoma tissue identified 10 clusters and 11 cell types, with OSR2 expression mainly in clusters 4, 8, and 10,

enriched with cDC1 cells, B-lymphocytes, mast cells, and naïve T cells (Fig. 1). Subcellular analysis using the HPA database and immunofluorescence on A-431 cells indicated OSR2 is primarily cytoplasmic. The Monaco database revealed high OSR2 expression in monocytes and B lymphocytes, but low in T and NK cells. The varied expression of OSR2 in dendritic cells, macrophages, and certain T-cell subsets, first observed in bulk data, was confirmed by HPA single-cell data. Further analysis using single-cell RNA sequencing and the CIDE database's Lineage function revealed a dynamic OSR2 expression pattern in both myeloid and lymphoid progenitors and their differentiated cells, such as dendritic cells, B cells, and monocytes, indicating its origin in immune cell lineages (Fig. 2).

3.2 Differential expression and prognostic significance of OSR2

Our analysis found that OSR2 was significantly upregulated in 13 cancer types, including glioblastoma multiforme (GBM) and glioma, and downregulated in 19 others, such as kidney renal papillary cell carcinoma (KIRP) and BLCA. Survival analyses for Overall Survival, Disease-Specific Survival, Disease-Free Interval, and Progression-Free Interval showed statistically significant results. Our analysis revealed that OSR2 expression was significantly dysregulated in tumor tissues and held prognostic value in seven cancer types, including lower-grade glioma (GBMLGG) and KIRP. OSR2 levels also showed a significant correlation with patient gender in six other cancers, such as breast invasive carcinoma (BRCA) and KIRC. Beyond prognosis and gender, OSR2 expression was linked to age across various cancers. In BLCA and KIPAN, higher OSR2 levels were associated with advanced tumor stage, overall stage, and grade, suggesting a role in tumor aggressiveness (Fig. 3).

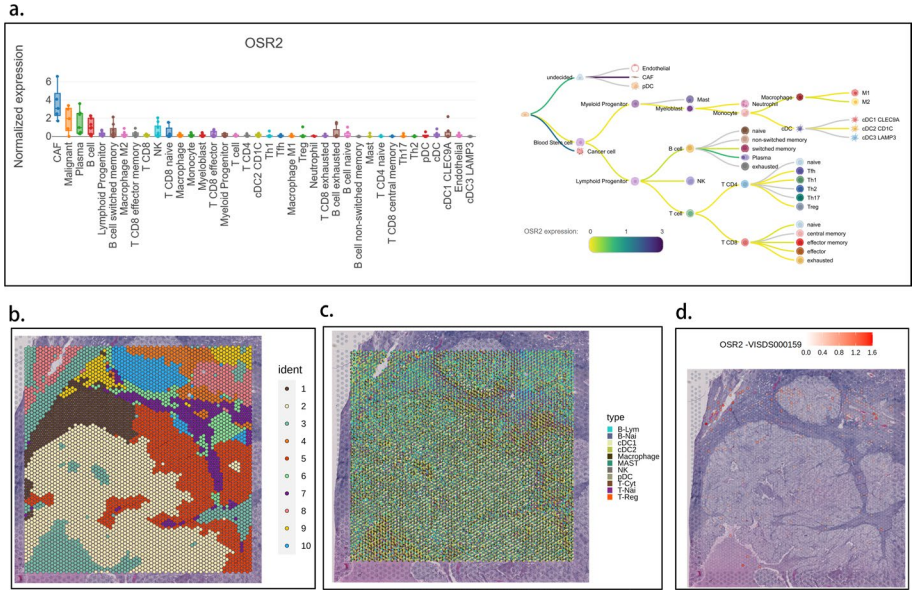

Fig. 1 OSR2 expression in renal carcinoma via single-cell and spatial transcriptomics (title). **a** depicts the standardized expression levels of OSR2 across various cell types. **b** provides a spatial distribution map of cells, indicating their regional localization within renal carcinoma tissue. **c** offers a micrograph of the tissue, corresponding to the ten identified cell clusters derived from the spatial transcriptomic analysis of the renal carcinoma sample. **d** illustrates the expression levels of OSR2 within the renal carcinoma tissue using a heatmap, emphasizing its expression patterns across eleven distinct cell types

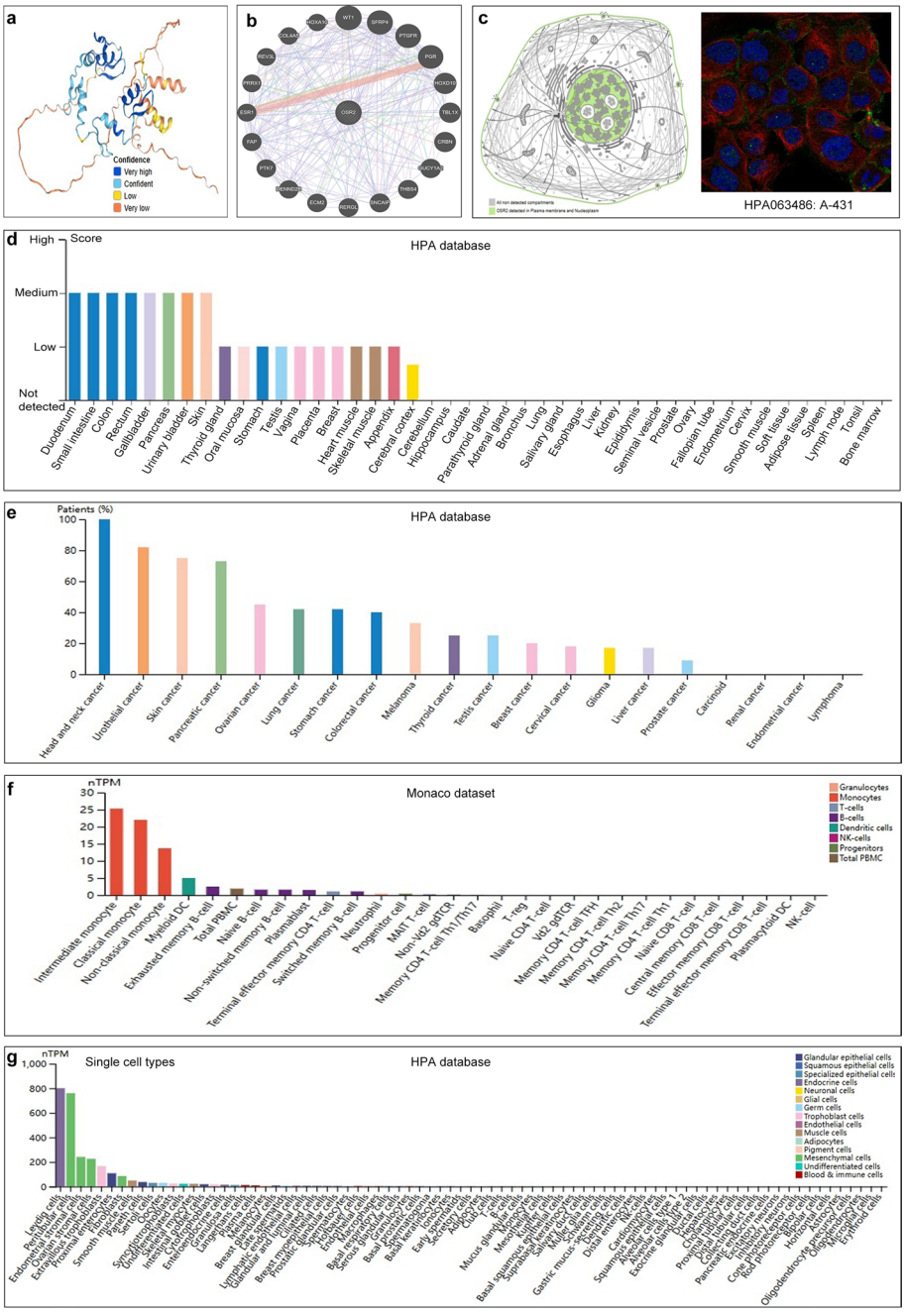

Fig. 2 OSR2 protein structure, interaction network, and tissue-specific expression (title). **a** depicts the predicted structural configuration of the OSR2 protein. **b** illustrates the protein-protein interaction network of OSR2, as analyzed using the GeneMANIA database. **c** provides a schematic representation of cellular architecture, highlighting the subcellular localization of the OSR2 protein. **d** presents the distribution of protein detection scores for OSR2 in normal tissue samples, derived from the Human Protein Atlas (HPA) database. **e** displays the proportion of patients exhibiting positive OSR2 detection across various cancer types, based on HPA database data. **f** illustrates the standardized expression levels (nTPM) of OSR2 across diverse immune cell subsets, as analyzed using the Monaco dataset. **g** presents the distribution of nTPM values for OSR2 in multiple cell types, including fibroblasts and neurons, based on analyses from the HPA Single Cell Type database

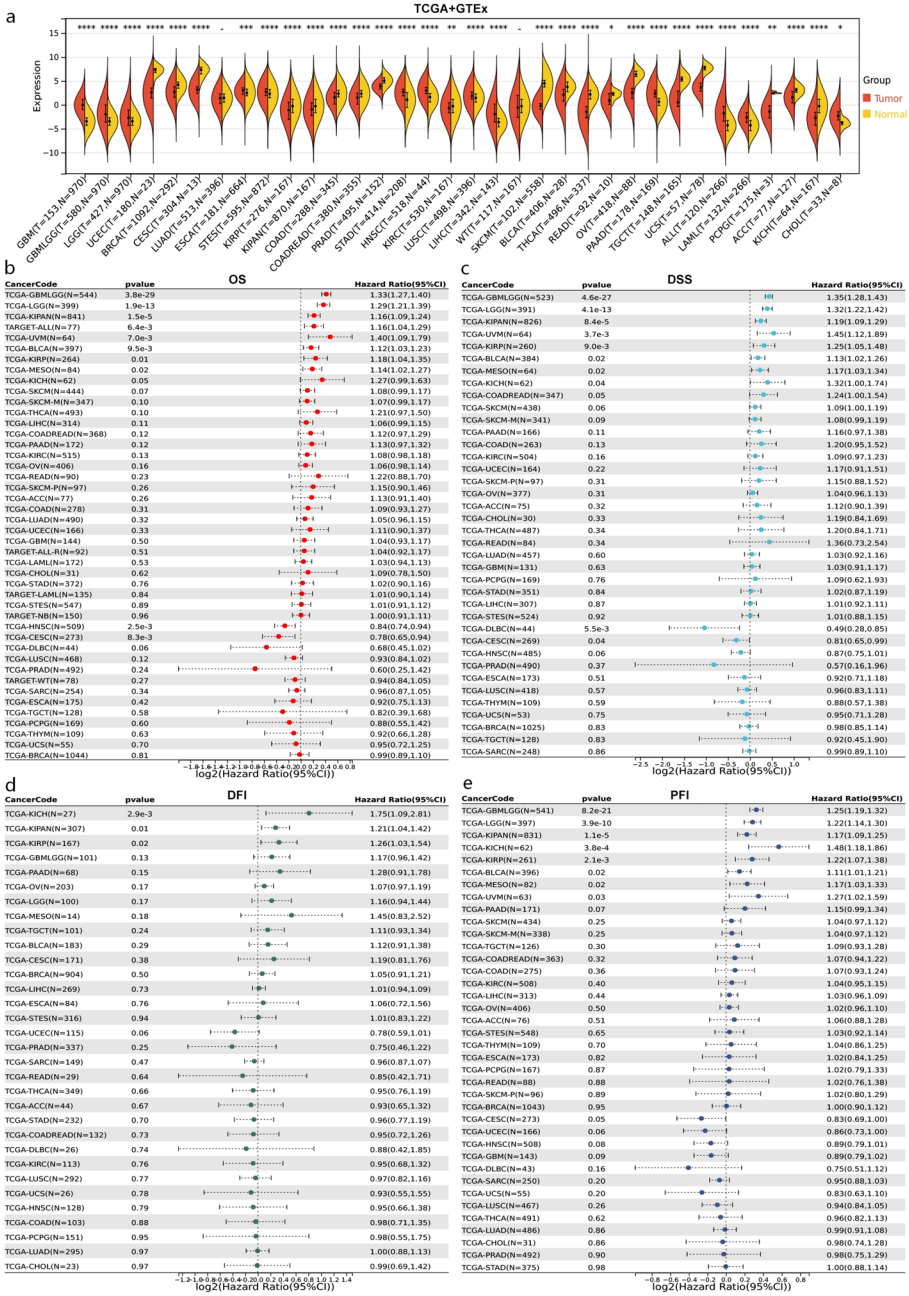

Fig. 3 OSR2's pan-cancer expression and prognostic value (title). **a** illustrates a comparative analysis of OSR2 expression levels between TCGA tumor samples and GTEx normal tissue samples across various cancer types, including GBM, LGG, and LUAD. Figure **b** which examines overall survival, demonstrates that elevated OSR2 expression (hazard ratio [HR] > 1) is significantly linked to poor prognosis in cancers such as GBMLGG, LGG, and KIPAN. **c** focusing on disease-specific survival, indicates that increased OSR2 expression is significantly correlated with reduced DSS in cancers including GBMLGG, LGG, and KIPAN. **d** concerning disease-free interval, shows that higher OSR2 expression is associated with a shorter DFI in cancer types such as KICH and KIPAN. Finally, **e** addressing progression-free interval, reveals that elevated OSR2 expression is significantly related to decreased PFI in cancers such as GBMLGG, LGG, and KIPAN

3.3 Tumor heterogeneity and stemness

The study found that OSR2 is linked to tumor heterogeneity through its correlation with TMB in nine cancer types. Seven cancers, including lung adenocarcinoma (LUAD) and colorectal adenocarcinoma (COAD), showed a strong positive correlation, while

sarcoma (SARC) and head and neck squamous cell carcinoma (HNSC) had a negative correlation (Fig. 4a). Regarding microsatellite instability (MSI), OSR2 mRNA expression was significantly correlated in eleven cancer types. Positive correlations were found in seven cancers, such as GBM and COAD, whereas negative correlations were noted in four types, including glioblastoma and GBMLGG and kidney renal papillary cell carcinoma (KIPAN).OSR2 mRNA expression showed significant associations with

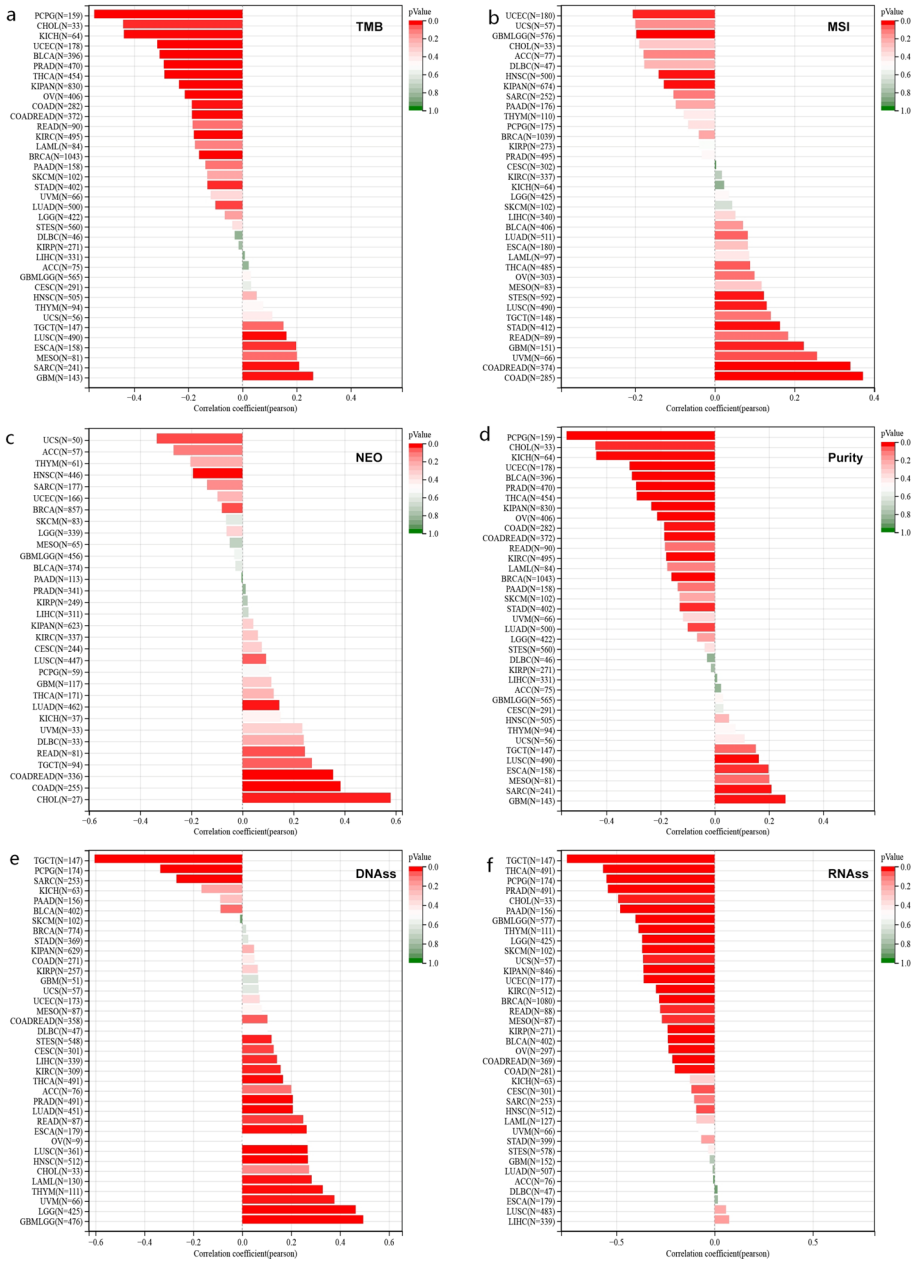

Fig. 4 OSR2 Expression's Link to Tumor Heterogeneity and Stemness(title). **a** depicts the correlation between OSR2 expression and TMB across 33 different cancer types. **b** illustrates the association of OSR2 with MSI. **c** provides a summary of the number of cancer types in which OSR2 demonstrates significant correlations with both TMB and MSI. Figure **d** presents the correlations between OSR2 and two stemness indices, DNAss and RNAss, across the same 33 cancer types. **e** and **f** further elucidate these relationships through scatter plots with linear regression curves, specifically emphasizing the linear association between OSR2 and DNAss in cancers such as GBM and LGG in (**e**) and the linear association with RNAss in cancers including KIRC and THCA in (**f**)

neoantigen load (NEO) and tumor purity across eight cancer types, with positive correlations in five types, including LUAD and COAD, and negative correlations in three types, such as BRCA and HNSC. Additionally, OSR2 mRNA expression was significantly correlated with the DNAss in 19 cancer types, displaying positive correlations in 16 and negative in three.

3.4 Gene mutations and RNA modifications

We analyzed the mutational status of OSR2 in various cancers and identified the top 20 types with the highest mutation frequencies, as shown in Fig. 5a. Bladder cancer and cervical squamous cell carcinoma had mutation frequencies of 0.7%, HNSC had 0.4%, and LGG had 0.2%. Using the median OSR2 expression, tumor patients were divided into high- and low-expression groups. We then examined the top 10 genes with the highest mutation frequencies in BLCA, CESC, DLBC, HNSC, KICH, KIRP, LGG, and MESO. The OSR2 expression groups showed distinct mutational patterns depending on the cancer type. In BLCA, high OSR2 expression correlated with mutations in KDM6A, RB1, and FGFR3, while in LGG, the mutations varied in TP53, IDH1, and EGFR.

3.5 Correlation analysis of OSR2 expression with immune modulator genes, immune checkpoint genes, and immune Cells

We systematically evaluated the co-expression patterns between OSR2 and 150 immune modulator genes, including chemokines, receptors, MHC molecules, immunoinhibitors, and immunostimulators. Our analysis showed that OSR2 positively correlates with most immune modulator genes in GBMLGG, BLCA, COAD, and KIPAN, but negatively correlates with them in TGCT. This suggests that OSR2 may have context-dependent and potentially opposing roles in immune regulation across various cancer types. We analyzed 60 immune checkpoint genes, dividing them into 24 inhibitory and 36 stimulatory types. OSR2 generally showed a negative correlation with immune checkpoints in KIPAN, BLCA, COAD, and GBMLGG, but a strong positive correlation in TGCT and LUSC. Additionally, OSR2 expression was inversely related to immune cell infiltration in PRAD, LGG, and PAAD, suggesting it may suppress immune cell recruitment in these cancers (Fig. 6).

3.6 Analysis of OSR2 expression in relation to RNA modification-associated genes, signaling pathways, and drug sensitivity

Our research found a strong link between OSR2 expression and RNA modifications (m1A, m5C, m6A) in various cancers, including PAAD, KIPAN, OV, and STAD. We also used the GDSC and CTRP databases to identify drugs that target tumors with high OSR2 expression, analyzing OSR2 expression and drug sensitivity across a pan-cancer cohort in the GDSC database. Our study found that OSR2 expressions significantly influence sensitivity to various targeted therapies. Specifically, it negatively correlates with Afatinib (an EGFR/HER2 inhibitor) but positively correlates with Dabrafenib and PLX4720 (both BRAF inhibitors). We also noted a negative correlation with Afatinib and a positive one with the HDAC inhibitor Entinostat in the CTRP database's top 30 drugs. Gene Set Variation Analysis (GSVA) showed that OSR2 expression is linked to key oncogenic pathways like autophagy, cell cycle regulation, and DNA damage response (Fig. 7).

Fig. 5 OSR2 mutation patterns in various cancers (title). **a** illustrates the distribution of OSR2 mutation frequencies across 20 distinct cancer types. **b** through I provide a comparative analysis of gene mutation profiles among patients categorized into high- and low-OSR2 expression groups, determined by the median expression value. This analysis is concentrated on eight specific cancer types: BLCA, CESC, DLBC, HNSC, KICH, KIRP, LGG, and MESO

4 Discussion

Recent advancements in cancer research have elucidated that genetic mutations and the activation of specific signaling pathways within tumor cells can substantially alter the tumor immune microenvironment, thereby enhance tumor heterogeneity and facilitate disease progression [29–31]. This study explores the cellular origin and spatial organization of OSR2 in the TME using single-cell transcriptomics and spatial transcriptomics. scRNA-seq reveals that the expression of OSR2 is primarily confined to the stromal compartment, particularly in CAFs, with limited expression in endothelial cells, while

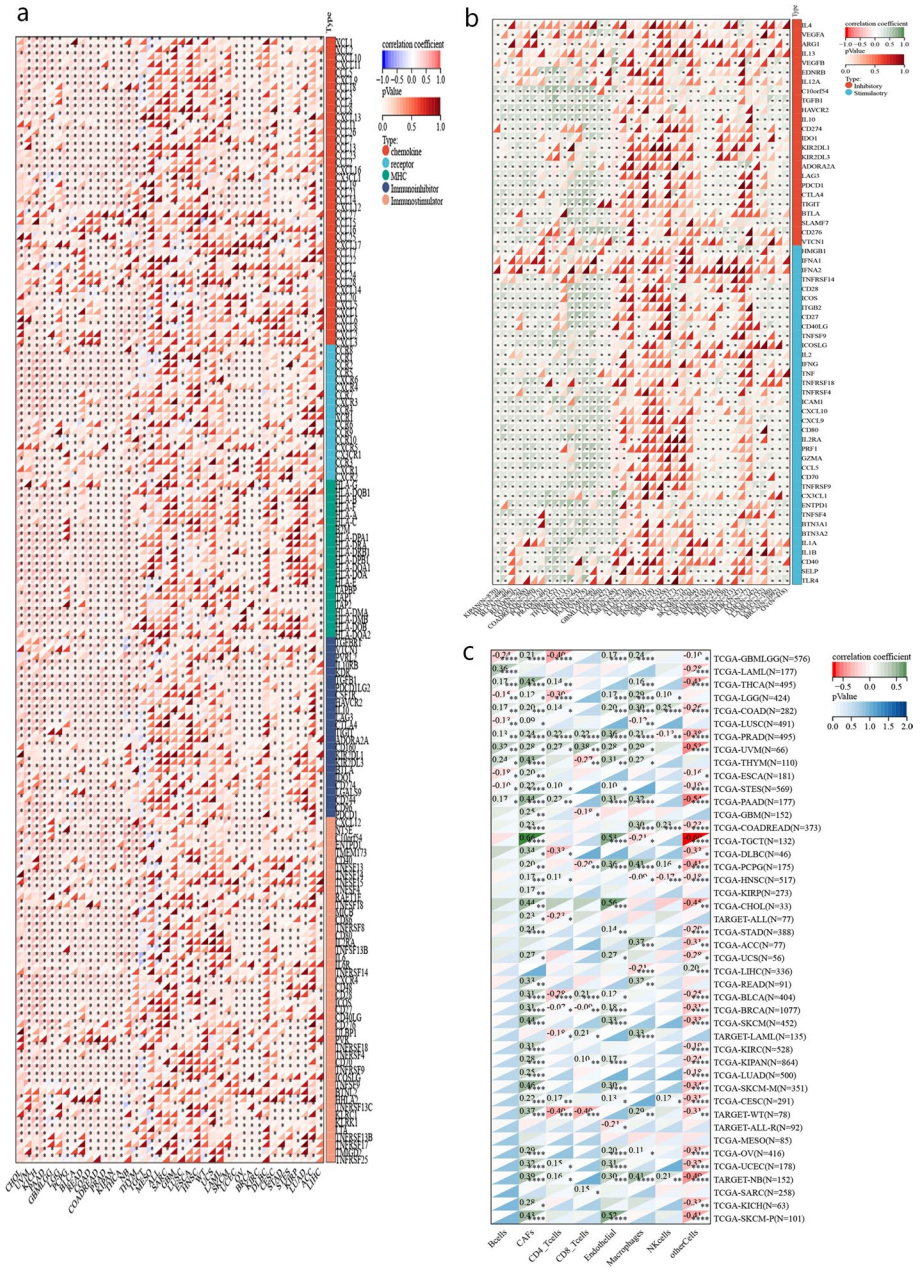

Fig. 6 OSR2's role in RNA modification, signaling, and drug sensitivity (title). **a** depicts a heatmap demonstrating the correlation between OSR2 expression and 44 RNA modification genes across various cancer types, with the rows annotated by the names of the RNA modification genes. **b** illustrates the association of OSR2 expression with 10 cancer-related signaling pathways, such as apoptosis, cell cycle, and EMT, as evaluated through GSVA. **c** examines the relationship between drug sensitivity in the CTRP database and OSR2 mRNA expression levels.

its expression in immune cell populations is minimal. Spatial transcriptomics further indicate that regions enriched in OSR2 are preferentially located in the stroma-dense areas at the tumor margin. Stromal compartments are increasingly recognized as critical regulators of antitumor immunity in solid tumors, in part through their capacity to organize the extracellular matrix and establish biomechanical and biochemical barriers that shape immune cell trafficking [32, 33]. Within this framework, OSR2-associated stromal regions may contribute to controlling immune access by influencing whether immune cells infiltrate the tumor parenchyma or remain confined to peripheral immune-active

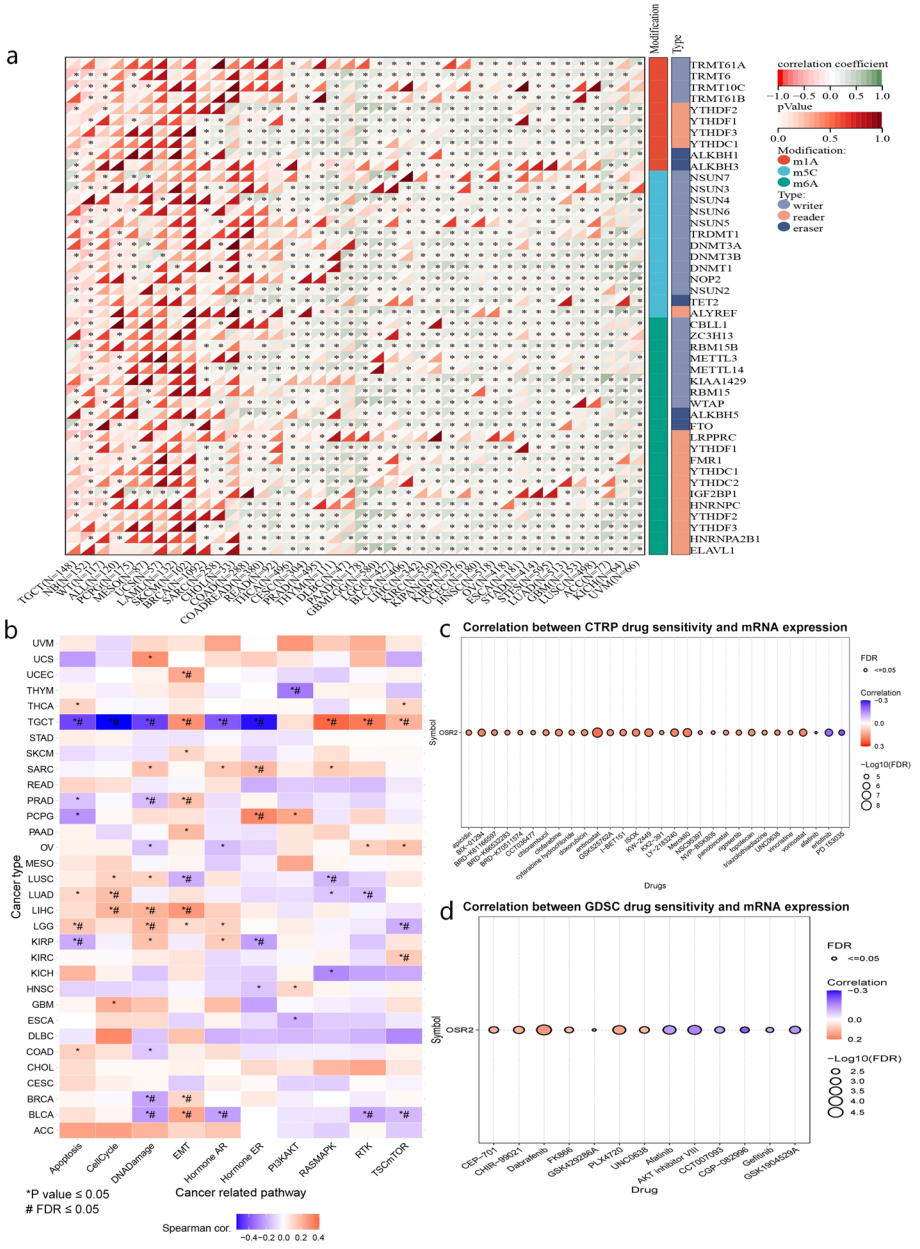

Fig. 7 OSR2's role in RNA modification, signaling, and drug sensitivity (title). **a** depicts a heatmap demonstrating the correlation between OSR2 expression and 44 RNA modification genes across various cancer types, with the rows annotated by the names of the RNA modification genes. **b** illustrates the association of OSR2 expression with 10 cancer-related signaling pathways, such as apoptosis, cell cycle, and EMT, as evaluated through GSVA. **c** examines the relationship between drug sensitivity in the CTRP database and OSR2 mRNA expression levels.

niches. These observations are aligned with ongoing translational efforts that exploit biomimetic/bioinspired nanoplatforms to enhance antigen delivery and immune priming [34, 35].

Numerous oncogenes and tumor suppressors demonstrate paradoxical, context-dependent functions. SIRT1 exemplifies this dualistic nature, acting as a genomic guardian by promoting DNA repair while also potentially facilitating tumor progression in certain cellular environments [36]. Our pan-cancer analysis revealed a distinct transcriptional dysregulation of the OSR2 gene, with significant upregulation in 13 cancer

types, particularly in gliomas like GBM and LGG. Conversely, OSR2 is downregulated in 19 cancers, such as kidney and bladder cancers, indicating its dual role as a tumor suppressor or promoter depending on the cancer type. OSR2's expression correlates with survival outcomes, showing tissue-specific effects, and suggests its potential as a dual-directional prognostic biomarker, warranting further research into its mechanisms. Studies show that genome-wide mutations in histone and DNA methylation genes significantly relate to intratumoral heterogeneity [37]. The tumor stemness can be examined using multi-omics data to understand tumor cell behaviors across cancer types [38]. Our study identified strong associations between OSR2 expression and tumor heterogeneity and stemness indicators. OSR2 exhibited a bidirectional relationship with TMB, being positive in some cancers (e.g., lung and colorectal adenocarcinomas) and negative in others (e.g., sarcoma and head and neck cancers). Additionally, OSR2 correlated significantly with DNA methylation-based stemness scores in 19 cancer types and RNA expression-based stemness scores in 24 types, mainly showing positive correlations. These findings suggest OSR2 may facilitate tumor progression by sustaining stem cell-like characteristics. The mutational landscape in cancer research is complex, involving genetic changes across various cancers and their roles in tumorigenesis. This study found that mutations occur in both coding and non-coding regions [39], with non-coding mutations heavily impacting gene regulation. OSR2 exhibited high mutation rates in various cancers, including 0.7% in bladder cancer. Variations in gene mutations, such as KDM6A and RB1 in bladder cancer and TP53 and IDH1 in lower-grade glioma, were noted among groups with different OSR2 expression levels, indicating these mutations could impair OSR2 function. The interaction between tumor genotype and immune phenotype can reveal mechanisms of immune evasion, while immune cell infiltration patterns in the tumor microenvironment may predict immune checkpoint inhibitor therapy success [40, 41]. In our pan-cancer immune correlation analyses, OSR2 displayed a clear context-dependent association with immune regulation. Specifically, OSR2 was positively correlated with most immune modulator genes in GBMLGG, BLCA, COAD and KIPAN, whereas an opposite pattern was observed in TGCT. These findings suggest that the functional significance of OSR2 as an immunological marker is heterogeneous across tumor types. Its expression pattern does not reflect a uniform state of immune activation or suppression but rather is associated with specific immunophenotypes that depend on the local microenvironment and the specific cell population in which the molecule is expressed. Importantly, OSR2 expression is positively linked to immune modulator genes in GBMLGG, BLCA, COAD, and KIPAN, but negatively associated with immune checkpoint genes in KIPAN and BLCA. The negative association between OSR2 and checkpoint genes observed in KIPAN and BLCA may reflect a relatively less inflamed immune context, while positive associations seen in other tumor types may arise in settings with greater immune engagement. In PRAD, LGG, and PAAD, OSR2 negatively correlates with immune cell infiltration. One plausible explanation is that OSR2-high tumors in these settings may exhibit features associated with reduced immune recruitment. Notably, our renal carcinoma spatial transcriptomics further supports the spatial heterogeneity of OSR2 within the tumor architecture.

In the field of cancer biology, RNA modifications are crucial for regulating RNA stability, translation, and molecular interactions, thereby impacting essential cellular processes, including the cell cycle and signaling pathways [42–45]. Importantly, regulators

of m6A modification are linked to the tumor immune microenvironment, stemness indices, and responsiveness to anticancer drugs, underscoring their significant prognostic value across various cancer types [46, 47].This study found that OSR2 expression is significantly linked to m1A, m5C, and m6A modification genes in PAAD, KIPAN, OV, and STAD, indicating its role in epigenetic regulation and potential prognostic use.

From a translational standpoint, the association between OSR2 expression and drug sensitivity across datasets suggests that OSR2 may serve as a stratification marker for therapeutic vulnerability in specific contexts. We analyzed drug sensitivity data from the GDSC and CTRP databases to evaluate OSR2's therapeutic significance. Our results show that high OSR2 expression predicts sensitivity or resistance to various targeted and chemotherapeutic drugs, suggesting OSR2 as a potential biomarker for drug efficacy and a target for therapy development. Autophagy-related genes exhibit considerable variability in expression across different cancer types, and the integrated autophagy-related signature score derived from these genes functions as a quantitative metric with substantial prognostic and predictive capabilities [48]. Additionally, cell cycle activity is intricately linked to DNA mutations and chromosomal aneuploidy, which collectively drive tumor progression and influence clinical outcomes [49]. Moreover, epithelial-mesenchymal transition is intricately linked with the increased activity of various developmental signaling pathways and immune activation, potentially facilitating disease progression through modulation of the tumor immune microenvironment [50, 51]. This study's signaling pathway analysis revealed a strong link between OSR2 and autophagy, cell cycle, and EMT pathways in cancers like bladder cancer and testicular germ cell tumors, highlighting its potential role in promoting tumors.

This study relies on bioinformatic predictions without wet-lab validation, particularly concerning OSR2's interaction pathways and synergy with RNA-modifying enzymes. To clarify OSR2's oncogenic or tumor-suppressive roles, future research should employ functional methods. Notably, although the pan-cancer analyses highlight the potential of OSR2 as a biomarker for personalized cancer therapy, the translational implications of targeting immune-associated genes must be interpreted cautiously. Immune-based interventions are increasingly accompanied by immune-related adverse events [52]. Therefore, further investigations integrating functional validation and clinical cohorts will be essential to determine whether OSR2 can serve as a target, particularly as immune checkpoint–related targets are increasingly being advanced toward precision medicine [53].

5 Limitation

Although we conducted a comprehensive bioinformatics analysis for the pan-cancer analysis of OSR2, there are still some limitations that need to be clarified. Our data is based on public databases; however, the sample size for certain tumors is limited, and the depth of clinical annotations varies. Batch effects and heterogeneity can impact the results across cohorts. Furthermore, the causal relationship of OSR2 at the molecular level still requires validation through in vitro and in vivo experiments. Lastly, the spatial transcriptomic analysis in this study focuses solely on specific renal cancer samples, which may limit the generalizability of the results. Future research should utilize larger spatial cohorts for validation.

6 Conclusion

This research identifies OSR2 as a potential prognostic biomarker and target for immunotherapy in various cancers. It shows that OSR2 is differentially expressed, with high levels linked to poor prognosis. OSR2 is associated with immune regulation, checkpoint genes, and immune cell infiltration, indicating its role in immune evasion and therapy response. This study provides a basis for future clinical applications and immunotherapeutic strategies.

Abbreviations

BLCA	Bladder cancer
TCR	T-cell receptor
OS	Overall survival
DSS	Disease-specific survival
PFS	Progression-free survival
scRNA-seq	Single-cell RNA sequencing
DFI	Disease-free interval
PFI	Progression-free interval
ANOVA	Analysis of variance
MSI	Microsatellite instability
NEO	Neoantigen
TMB	Tumor mutation burden
DNAss	DNA methylation-based stemness scores
RNAss	mRNA-based stemness scores
GDC	Genomic Data Commons
NK	Natural killer
CAFs	Cancer-associated fibroblasts
GBM	Glioblastoma multiforme
KIRP	Kidney renal papillary cell carcinoma
BRCA	Breast invasive carcinoma
LUAD	Lung adenocarcinoma
COAD	Colorectal adenocarcinoma
SARC	Sarcoma
HNSC	Head and neck squamous cell carcinoma
MSI	Microsatellite instability
KIPAN	Kidney renal papillary cell carcinoma
TAMs	Tumor-associated macrophages

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s12672-026-04595-z.

Supplementary Material 1.

Acknowledgements

The authors utilized AI-assisted editing tool (Grammarly and ChatGPT-4) solely for language refinement purposes, including grammar checking, sentence structure optimization, and vocabulary enhancement. All conceptual development, technical content, and critical analysis remain entirely human-generated. The authors take full responsibility for the accuracy of information and academic integrity of this work.

Author contributions

ZHH proposed the project, conducted data analysis, interpreted the data, and wrote the manuscript; ZHH, HHY, FLS and DCF conducted data analysis, interpreted the data; FLS, DCF and WRW supervised the project, and interpreted the data. All authors reviewed and edited the manuscript.

Funding

None.

Data availability

In this study, we utilized the following datasets and repositories for our analyses: Single-cell RNA sequencing (scRNA-seq) analysis: We employed the Lineage function of the Cancer Immunotherapy Data Explorer (CIDE) database to analyze the target gene's expression across various immune cells and identify its cellular origins. The dataset can be accessed directly via the following link: https://cide.ccr.cancer.gov.Comprehensive repository of spatial transcriptomics (CROST) database (https://ngdc.cncb.ac.cn/crost/home) was used for spatial transcriptomics analysis. The data (VISDS000159) was selected for further analysis. Spatial plots were used to show the expression levels in the kidney cancer.OSR2 gene and protein analysis: A comprehensive analysis of OSR2 (ENSG00000164920) was performed using the Human Protein Atlas database. The specific dataset for OSR2 can be accessed via the following link: https://www.proteinatlas.org/ENSG00000164920-OSR2. Additionally, we utilized the GeneMANIA database to explore OSR2 gene interactions and functional associations. The dataset is available here: https://genemania.org/search/homo-sapiens/OSR2.Pan-cancer dataset analysis: We extracted

OSR2 (ENSG00000164920) data from the standardized pan-cancer dataset TCGA TARGET GTEx (PANCAN, N=19,131, G=60,499), which was obtained from the UCSC Xena database. The dataset can be accessed via the following link: https://xena.ucsc.edu.EPIC analysis: The deconvo_epic method from the R package IOBR (version 0.99.9) was used to evaluate immune cell infiltration. Recalibration was applied to the gene expression dataset to enhance infiltration scores of key immune and stromal cell types.Immune Landscape of Cancer analysis: The standardized pan-cancer dataset TCGA Pan-Cancer (PANCAN, N=19,131, G=60,499) was downloaded from the UCSC Xena platform (https://xenabrowser.net/). Expression data for ENSG00000164920 (OSR2) and 150 immune pathway marker genes across five categories—chemokine (41 genes), receptor (18 genes), MHC (21 genes), immunoinhibitor (24 genes), and immunostimulator (46 genes)—were extracted for further analysis.GSCALite, GDSC, and CTRP analysis: The GSCALite platform (http://bioinfo.life.hust.edu.cn/web/GSCALite/) was used for a dual-faceted analysis. First, correlations between OSR2 expression and cancer-related signaling pathway activities were systematically evaluated. Second, by integrating pharmacogenomic data from the GDSC and CTRP databases within GSCALite, the relationship between OSR2 expression levels and sensitivity to anticancer drugs was examined.All datasets were accessed on August 1, 2025.

Declarations

Ethics approval and consent to participate

This study was conducted using publicly available data from the public databases. All data used in this study were de-identified and previously published. Therefore, no additional ethical approval was required. Not applicable. This study did not involve direct participation of human subjects, and all data were obtained from publicly accessible datasets.

Consent for publication

Not available.

Competing interests

The authors declare no competing interests.

Received: 20 October 2025 / Accepted: 3 February 2026

Published online: 09 February 2026

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