Anesthesiology and
Perioperative Science

ORIGINAL RESEARCH

Expression heterogeneity, tumor immune characteristics and the prognosis effects of *OPRL1* in patients with tumors: a pan-cancer study combined with bioinformation analyses and in vitro validation

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Abstract

Purpose Opioids are currently the most frequently prescribed analgesics in clinical practice. However, their effect on cancer progression remains a topic of debate. Opioid receptors (ORs) are present in various types of tumor cells and their expression levels vary depending on the type of tumor. This study aims to explore and preliminarily characterize the association between four different ORs (μ, δ, κ, and nociception/orphanin FQ peptide receptor) and the prognosis of different types of tumors for comparison, with a focus on nociception/ orphanin FQ peptide receptor.

Methods The expression levels of four ORs in normal tissues and immune cells were obtained from Human Protein Atlas (HPA) RNA-seq dataset, Monaco dataset, and Consensus dataset. Pan-cancer analysis was performed using the The Cancer Genome Atlas (TCGA) dataset, which included the expression of four ORs in different cancer types, significant copy-number alterations (sCNA), gene mutations of the four ORs, survival analysis, co-expression genes analysis, functional enrichment analyses, and correlations between ORs and immune cell infiltration levels. Based on the results of bioinformatic analysis, we selected 10 cancer cell lines for validation in vitro using specific agonists for the four ORs.

Results *OPRL1* (opioid related nociceptin receptor 1 gene) exhibited the highest abundance across different types of cancers, while *OPRM1* (opioid receptor mu 1 gene) and *OPRD1* (opioid receptor delta 1 gene) were barely detectable in multiple cancer types. Pan-cancer survival analysis revealed the overall worse/better prognosis of the four ORs in certain cancer types. Elevated levels of *OPRM1* appear to be associated with poorer outcomes in breast invasive carcinoma and kidney renal clear cell carcinoma. Elevated *OPRD1* levels are connected to worsen outcomes in kidney renal clear cell carcinoma and liver hepatocellular carcinoma, but better prognosis in bladder urothelial carcinoma. Increased *OPRK1* (opioid receptor kappa 1 gene) expression is linked to a poorer prognosis in kidney renal papillary

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cell carcinoma. Furthermore, high *OPRL1* expression relates to worse outcomes in bladder urothelial carcinoma and liver hepatocellular carcinoma, but better outcomes in breast invasive carcinoma and pancreatic adenocarcinoma. Functional enrichment analyses found that immune-related pathways were enriched in *OPRK1* and *OPRL1*, with *OPRL1* exhibiting the highest correlation with immune cell infiltration. Different effects on cell growth, migration, and invasion were observed in different cancer types upon the administration of agonists for the four ORs.

Conclusion *OPRL1* may play a vital role in monocytes and regulating the immune response and tumor-infiltrating macrophages. Due to its high abundance in different types of tumors, it may hold greater clinical significance for oncology patients. *OPRK1* also participates in immune-related pathways. *OPRL1* could potentially serve as therapeutic targets for different types of cancers.

Keywords Opioid receptor, Cancer, Tumor immune microenvironment, OPRL1, Overall survival, TCGA

Graphical Abstract
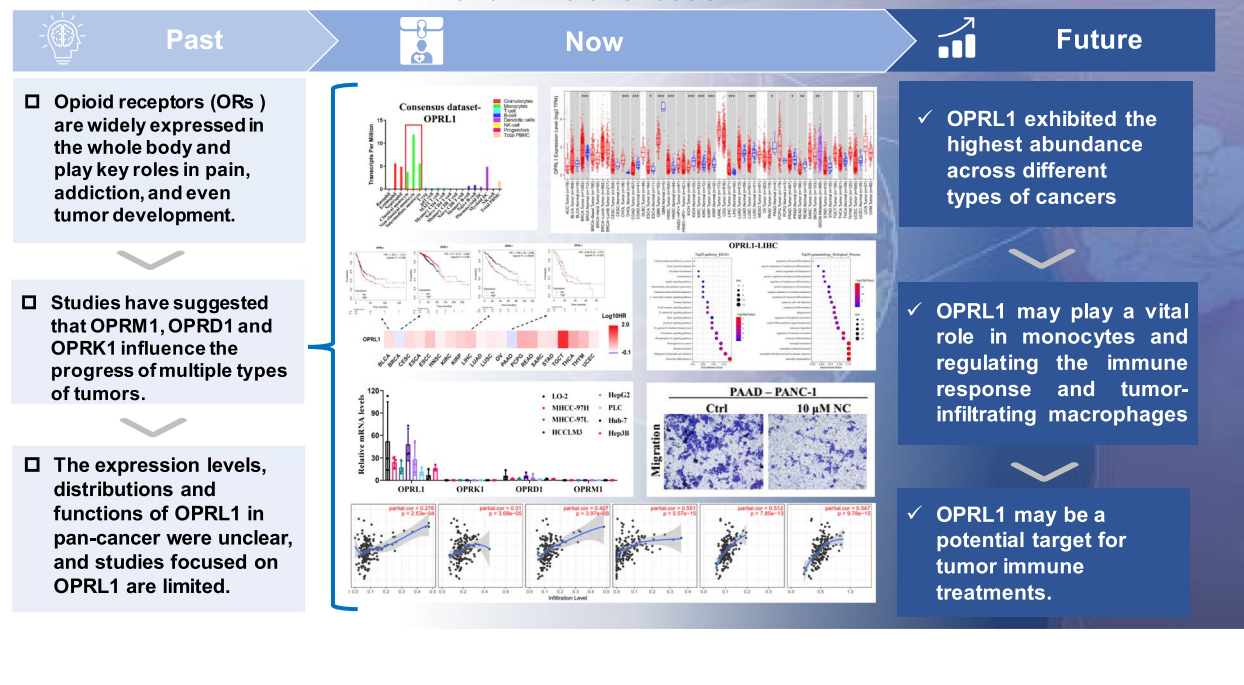
1 Introduction

Analgesia and pain management are essential components of cancer treatment, and there is growing evidence that pain can promote cancer progression and metastasis [1]. Opioids are commonly used for pain control due to their ability to modulate pain perception in the central and peripheral nervous systems. As one of the three pillars of anesthesia (alongside sedation and muscle relaxation), opioids are an integral part of clinical analgesia and anesthesia. They also influence cellular and humoral immune responses, as well as the expression of chemokines and chemokine receptors [2]. In current clinical practice, opioids are the most commonly prescribed analgesics during the perioperative period of cancer surgery, and they are often administered to patients with moderate to severe pain associated with advanced cancer or treatment-related complications [3].

To exert their effects, opioids must bind to opioid receptors, which are present on both neuronal and non-neuronal cells. The opioid system has been extensively studied for its roles in pain, reward, and addiction. Currently, there are four known ORs: μ opioid receptor (MOR, encoded by the *OPRM1*), δ opioid receptor (DOR, encoded by the *OPRD1*), κ opioid receptor (KOR,

encoded by the *OPRK1*), and the nociception/orphanin FQ peptide receptor (NOPR, encoded by the *OPRL1*) [4, 5].

The effect of opioids on cancer progression is still a matter of debate. Several experimental studies have suggested that morphine promotes proliferation, migration, and invasion in human breast cancer cells [6], clear cell renal cell carcinoma cells [7] and colorectal cancer cells [8], as well as stimulated angiogenesis in mouse breast cancer models [9, 10]. One small clinical cohort for naltrexone provided a proof of principle that opioid receptor antagonists have the potential to improve outcomes in breast cancer [11]. Preclinical studies also suggest that opioids may be associated with tumor progression and recurrence, which can have negative prognostic implications for cancer patients [12, 13]. A retrospective study found that opioid requirement remained an independent predictor of shorter survival in non-small cell lung cancer [14]. However, other studies have arrived at opposite conclusions, with some showing that opioids may even inhibit several cancer cell lines and animal models. For example, morphine significantly decreased the adhesion, invasion, and metastasis of metastatic colon cancer cells [15]; while fentanyl inhibited cell viability and invasion in lung [16] and gastric cancer [17]. Heterogeneity between existing studies, including differences in type of opioid, dose, and route of administration, different tumor cell lines and cancer models used may account for the varied effects of opioids. Also, there is a lack of guidance from the clinical trials on opioids and disease progression [18].

Studies have found that opioid receptors and peptides are widely distributed in various types of tumor cells and the expression levels differ according to the tumor tissue type [19]. Meanwhile, ORs can influence the release and function of cytokines and chemokines [20]. Some studies suggest that ORs may contribute to cancer metastasis since they are generally upregulated in metastatic samples compared to non-metastatic ones [21], and the ORs expression levels correlate to reduced overall survival and progression free survival in several cancers [22, 23]. There is also a connection between ORs and tumor immunity, although the available research evidence is insufficient. The tumor microenvironment (TME), which exerts a significant impact on cancer progression, constitutes a complex system consisting of cancer cells, endothelial and stromal cells, leukocytes, fibroblasts, and neurons that release soluble mediators triggering neuroinflammation [24]. The cellular components of the TME express MOR, which initiates signaling events that induce neuroinflammation in the presence of morphine. The activation of MOR not only affects pain and the quality of life

for cancer patients, but also directly and/or indirectly influences tumor growth and metastasis [20]. Opioids may modify the infiltration of immune cells into the TME [25]. In comparison to MOR-knockout mice, long-term administration of the same morphine to wild-type mice led to the atrophy of lymphoid organs and a reduced ratio of $CD4^+/CD8^+$ T cells in the thymus [26]. Additionally, MOR affects immunity through the hypothalamic-pituitary-adrenal axis, resulting in adrenocortical dysfunction and immunosuppression [27]. In this way, the overall effect of opioid agonism on cancer is far from trivial.

Contemporary research predominantly concentrates on µ or δ receptors in specific cancers, whereas the effects and functions of KOR and NOPR remain unclear in tumorigenesis. Additionally, the variances in expression levels, distribution characteristics, and connections with tumor immunity of the four ORs across diverse cancers have not been elucidated. Therefore, comprehending the overall perspective of the four ORs and their association with tumor development is crucial for providing anesthesia to surgical oncology patients and administering drugs to tumor patients. This study aims to acquire a comprehensive and systematically structured understanding of the correlation between the four ORs and the prognosis of different tumors. It primarily highlights the potential influence of the ORs on the tumor immune microenvironment (TIM). Furthermore, through this study, we have tentatively identified that NOPR, which has received less attention previously, may play a pivotal role in regulating the TIM and may represent a novel therapeutic target for tumors.

2 Methods

2.1 Expression and distribution of ORs in normal tissues and immune cells

The expression levels of four ORs in different normal tissues and immune cells derived from peripheral blood were acquired from three datasets: the HPA RNA-seq dataset [28, 29] (https://www.proteinatlas.org/), the Monaco dataset [30], and the Consensus dataset [28, 29, 31]. A total of 51 human tissues and 18 blood cell types were analyzed by RNA-seq to estimate the transcript abundance of each protein-coding gene in the HPA dataset. For the Monaco dataset, 29 immune cell types of healthy donors were characterized using RNA-seq and flow cytometry. The Consensus dataset comprised normalized expression levels for 55 tissue types, generated by merging the HPA and the Genotype-Tissue Expression dataset using the internal normalization pipeline [31]. All data was downloaded from the websites of the HPA and the Monaco dataset.

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2.2 Expression of four ORs in cancer and paired adjacent tissues

To investigate the expression levels and variations of four ORs across various cancer types, a pan-cancer analysis of OR expression levels was conducted using TCGA dataset (https://portal.gdc.cancer.gov/). TCGA is a pioneering program in cancer genomics that encompasses over 20,000 primary cancer and matched normal samples spanning 33 cancer types, and has been extensively utilized for tumor research. Additionally, graph visualization was performed using TIMER 2.0 (http://timer.comp-genomics.org/) [32] for graph drawing. The detailed full names of different cancers are supplied in the Supplementary Table 1.

2.3 sCNA and gene mutation of four ORs across TCGA

As two important gene structure variations, the sCNA and gene mutation data were acquired and collected from the TCGA dataset and graph drawing was completed by using the tool TIMER 2.0. Increasing evidence has shown that copy number alteration (CNA) plays an important role in the pathogenesis and prognosis of cancers [33], and we mainly compared five types of CNA in cancer: deep deletion, arm-level deletion, diploid/normal, arm-level gain and high amplication [34]. Similarly, gene mutation levels reflect cancer genomic stability and gene function deletion [33].

2.4 Survival analyses of four ORs in different types of cancers

A pan-cancer survival analysis was performed to explore effects of four ORs on the overall survival (OS) of patients with tumor using the Kaplan-Meier survival analysis, and all survival analyses were completed by using the tool Kaplan-Meier Plotter (http://kmplot.com/analysis/) [35]. The optimal cut-off value of four ORs genes were selected and the Log-rank test was used for comparing differences between low/high expression groups.

2.5 Co-expression genes analysis and functional enrichment analyses

The co-expression genes of ORs were obtained from the cBio Cancer Genomics Portal (cBioPortal, http://cbioportal.org) [36]. The cBioPortal website is a publicly accessible platform for interactive exploration of complex cancer genomics datasets. It offers access to data from over 30,000 tumor samples derived from 334 distinct cancer studies. Furthermore, specific cancer types which exhibited statistically significant survival differences were chosen for co-expression gene analyses. Specifically, the TCGA dataset (sourced from GDAC Firehose) [37] was utilized for identification and analysis of co-expression genes associated with these cancers.

To investigate the biological functions of ORs in various cancers, Gene Ontology (GO) enrichment analyses and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were conducted using ORs and their co-expression genes. The 300 most highly correlated co-expression genes were selected for functional enrichment analyses based on Spearman's correlation score, and the top 20 enriched pathways were visually represented in figures. GO can be accessed at http://geneontology.org/, and KEGG at https://www.kegg.jp/. All functional enrichment analyses were performed using the Sangerbox tools, a free online platform for data analysis (http://www.sangerbox.com/tool).

2.6 Correlations between ORs and immune cell infiltration levels

The correlations between ORs expression and immune cell infiltration levels were analyzed by the Tumor IMmune Estimation Resource (TIMER 1.0) web server (https://cistrome.shinyapps.io/timer/) [32, 38]. TIMER 1.0 is a comprehensive resource that facilitates systematic analysis of immune infiltrates across diverse cancer types. The abundance of six immune infiltrates (B cells, CD4^{+} T cells, CD8^{+} T cells, Neutrophils, Macrophages, and Dendritic cells) are estimated by the TIMER algorithm. For further analysis of immune cell infiltration, specific cancers exhibiting statistically significant survival disparities were selected.

2.7 Expression distributions of ORs in TME

To investigate the expression patterns of ORs within the TME, the authors utilized a tool called the Tumor Immune Single-cell Hub (TISCH) that facilitates exploration of TME across different single-cell transcriptomic analyses (http://tisch.comp-genomics.org/home/) [39]. TISCH enables detailed cell-type annotation at the single-cell level, providing insights into TME composition in various cancer types. Four datasets were chosen for analysis: BLCA_GSE145281, LIHC_GSE140228, BRCA_GSE114727, and PAAD_GSE111672.

2.8 Cell culture and drugs treatments

In total 18 cell lines were used for fundamental experiments: breast cancer (BRCA) cell lines, T47D and MDA-MB-231; bladder cancer (BLCA) cell lines,5637 and T24; Liver hepatocellular carcinoma (LIHC) cell lines, HCCLM3, MHCC-97H, MHCC-97L, HepG2, PLC, Huh-7 and Hep3B; Kidney renal clear cell carcinoma (KIRC) cell line, OS-RC-2; Kidney renal papillary cell carcinoma (KIRP) cell line, ACHN; Uterine Corpus Endometrial Carcinoma (UCEC) cell line, AN3CA; Pancreatic adenocarcinoma (PAAD) cell line, PANC-1; Monocyte cell line, THP-1; T cell line, Jurkat; Normal hepatic cell

line, LO-2. All cell lines were purchased from the FuHeng Cell Center (Shanghai, China) and authenticated by short tandem repeat tests before cell culture. All cell lines were cultured in high glucose Dulbecco's modified Eagle's medium (DMEM; Gibco BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; Gibco) except AN3CA. AN3CA was cultured with McCOY's 5A medium (Genomcell.bio, Zhejiang, China) supplemented with 10% FBS (Gibco). Also, 50 U/ml penicillin and 50 μg/ml streptomycin (Yeasen, Shanghai, China) were added into the medium regularly. Cells were culture in a 37 ℃ humidified incubator containing 5% CO_2. When cells reached 80%–90% confluency, they were detached with trypsin–EDTA (0.25%) (Yeasen) and used for subsequent experiments.

To test effects of ORs on cell proliferation, cell migration and invasion, specific agonists of ORs were used during cell culture: 1.[D-Ala2, N-Me-Phe4, Gly5-ol]-enkephalin (DAMGO), MOR agonist (Cat. No. ab120674; Abcam, Boston, MA, USA); 2. [D-Pen2,D-Pen5]-enkephalin, DOR agonist (Cat. No. ab120675; Abcam); 3. U69593, KOR agonist (Cat. No.ab141703; Abcam); 4. Nociceptin, NOPR agonist (Cat. No. ab120070; Abcam). The concentration of all four agonists for the experiments is 10 μM. DAMGO, DPDPE and nociceptin were diluted in phosphate buffered saline (PBS), and U69593 was diluted in dimethyl sulfoxide (DMSO) based on specifications. The same volume of PBS or DMSO were added to the control group. U69593 is a potent and selective KOR agonist, which is commonly used as a tool drug [40, 41].

2.9 Cell counting kit (CCK-8) assay
Cells were seeded into the 96-well plate at the density of 5×10^3 cells/well with 100 μl culture medium and corresponding agonists or not. After 48 h culture, 10 μl CCK-8 (Cat. No. CK04; Dojindo, Japan) was added into cells for 30 min incubation. Then, optical density (OD) at 450 nm was detected by the microplate reader (Bio-Rad, Hercules, CA, USA). The value of 450 nm OD represents the number of cells.

2.10 Transwell migration and invasion assays
Cell migration and invasion ability were detected by Transwell chambers (8-μm pore size, Corning, New York, USA) and Matrigel (only for invasion ability detection, Corning) according to previous protocols [42]. In migration assays, 1×10^5 cells/ml in 200 μl medium without FBS were added to the upper chamber. In invasion assays, the upper chambers were initially coated with 0.5 mg/L Matrigel for 30 min to form a membrane. The lower chambers contained 600 μl DMEM (with 10% FBS). Plates were incubated for 24 h, and then the chambers were fixed with 4% paraformaldehyde (Servicebio, Wuhan, China) for 20 min and stained with 0.5% crystal violet for 20 min. For both assays, three randomly selected fields per chamber were quantified using ImageJ 1.51 software to calculate averages reflecting the migration or invasion ability for each sample. All experiments were performed in triplicate. Digital images were captured using an Olympus fluorescent microscope equipped with bright field function.

2.11 RNA isolation and RT-qPCR analysis
Total RNA was isolated using EZ-press RNA Purification Kit (EZ Bioscience, USA) following the manufacturers' protocol. Reverse transcription of 0.5 μg of total RNA was performed using the reverse kit (EZ Bioscience). $2 \times$ SYBR Green qPCR Master Mix (EZ Bioscience, USA) was used for RT-qPCR, and the reaction conditions were as follows: pre-denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 10 s and 60 °C for 30 s. The Ct values obtained from the samples were calculated using the $2^{-\Delta\Delta Ct}$ method. The primers of genes were as follow (all human derived): *OPRM1*, forward 5'- GCCCTTCCAGAGTGTGAATTAC-3', reverse 5'- GTGCAGAGGGTGAATATGCTG-3'; *OPRD1*, forward 5'- CGTCCGGTACACTAAGATGAAGA-3', reverse 5'- GCCACGTCTCCATCAGGTA-3'; *OPRK1*, forward 5'- ATCATCACGGCGGTCTACTC -3', reverse 5'- ACT CTGAAAGGGCATGGTTGTA -3'; *OPRL1*, forward 5'- TTCTGGGAGGTTATCTACGGC-3', reverse 5'- GGA TGACGTACATGACAAGGC-3'; *GAPDH*, forward 5'- GGAGCGAGATCCCTCCAAAAT-3', reverse 5'- GGC TGTTGTCATACTTCTCATGG-3'.

2.12 Statistical analysis
Statistical analyses were conducted using the IBM SPSS Statistics 26.0 software (SPSS Inc., Armonk, New York, USA) and GraphPad Prism 7 software. All data were expressed as the mean ± standard deviation (SD), and differences were analyzed through independent samples t-test. P value < 0.05 is considered statistically significantly different.

3 Results
3.1 Expression and distribution heterogeneity of four ORs in different tissues and immune cells
Through the examination of ORs expression levels in diverse tissues from the Consensus dataset, it is indicated that ORs are predominantly expressed in the central nervous system, encompassing regions such as the cerebellum, basal ganglia, cerebral cortex, and amygdala (Fig. 1A). Furthermore, *OPRM1* displays high expression levels in the testis, *OPRD1* is highly expressed in skeletal muscle and granulocytes, *OPRK1* is highly expressed
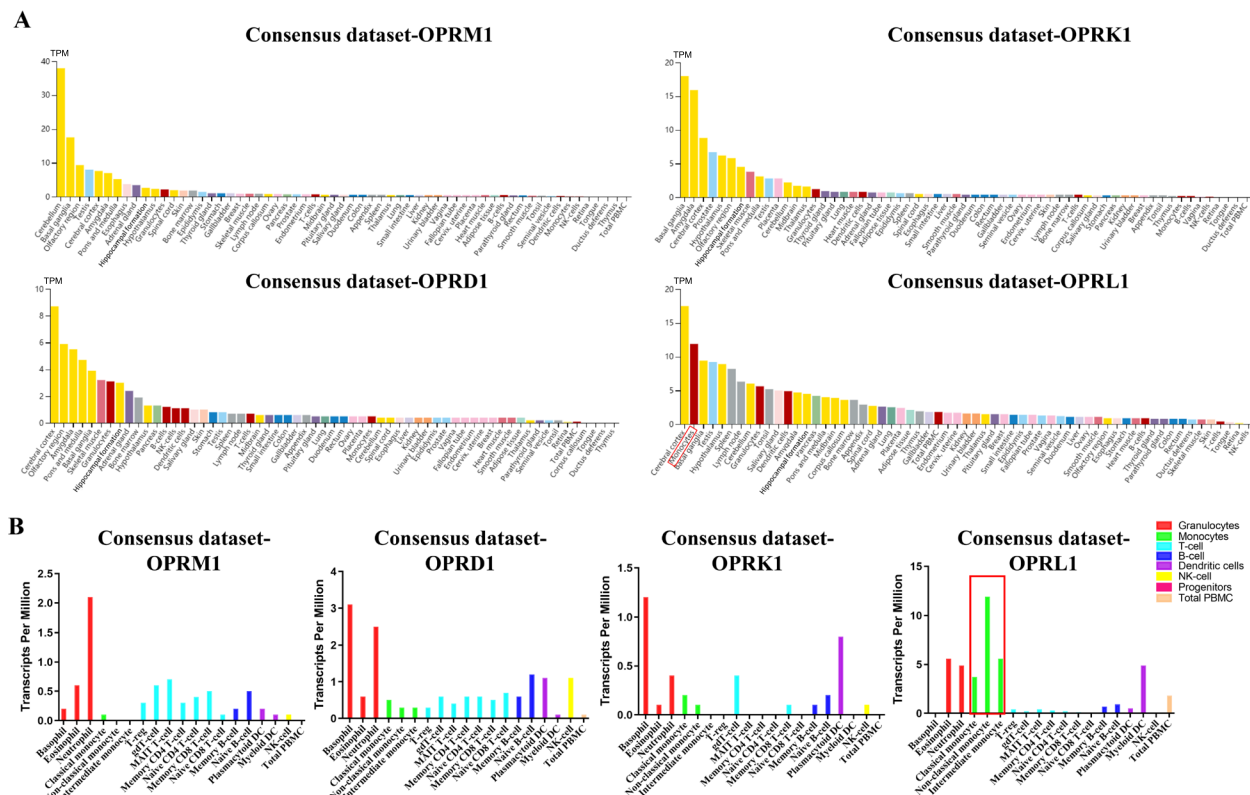
Fig. 1 Variations in expression and distribution patterns of four ORs across distinct tissues and immune cells. **A**: The Consensus dataset reveals that gene expression levels of the four ORs vary among different tissues, with ORs predominantly expressed in the central nervous system, while *OPRL1* displays high expression specifically in monocytes. **B**: Analysis of immune cells in the Consensus dataset shows that the four ORs are widely expressed and distributed. The expression level of *OPRL1* is significantly higher than that of the other three ORs, and it is mainly expressed in monocytes. ORs, Opioid receptors; OPRM1, opioid receptor mu 1 gene; OPRD1, opioid receptor delta 1gene; OPRK1, Opioid receptor kappa 1 gene; *OPRL1*, opioid-related nociceptin receptor 1 gene; PBMC, peripheral blood mononuclear cell; TPM, transcripts per million

in the prostate and testis, while *OPRL1* exhibits high expression levels in monocytes, testis, granulocytes, and dendritic cells (Fig. 1A & Supplementary Fig. 1A).

Subsequently, expression levels of four ORs across various immune cell types were comprehensively compared in the Consensus dataset, HPA dataset, and Monaco dataset (Fig. 1B & Supplementary Fig. 1B–C). The expression of ORs was found to be ubiquitous and present across several immune cell types. Furthermore, the findings indicate that *OPRL1* was primarily expressed in monocytes, eosinophils, neutrophils, and myeloid dendritic cells (Fig. 1B & Supplementary Fig. 1B–C). The expressions of OPRL1 in classical monocyte, non-classical monocyte and intermediate monocyte were 3.7, 11.9 and 5.6 respectively, while expressions of OPRM1 were 0.1, 0 and 0 respectively, expressions of OPRK1 were 0.2, 0.1 and 0 respectively, expressions of OPRD1 were 0.5, 0.3 and 0.3 respectively.

3.2 Expression changes and gene structure variations of four ORs in pan-cancer

The expression levels of four ORs were analyzed between tumor and normal tissue samples across the TCGA dataset. Pan-cancer analysis revealed that *OPRL1* could be detected across all types of cancers (Fig. 2). However, the expression levels of *OPRM1* and *OPRD1* were too low to be detected in multiple cancer types (Fig. 2). This analysis also showed significant changes in gene expression levels, with notable downregulation or upregulation of the four ORs observed across multiple cancers.

Furthermore, the sCNA and gene mutation levels of four ORs across TCGA cancer dataset were analyzed (Supplementary Fig. 2). The sCNA analysis showed contrasting patterns in the composition of the four ORs, with more arm-level deletions observed in *OPRM1* and *OPRD1*, while more arm-level gains and high
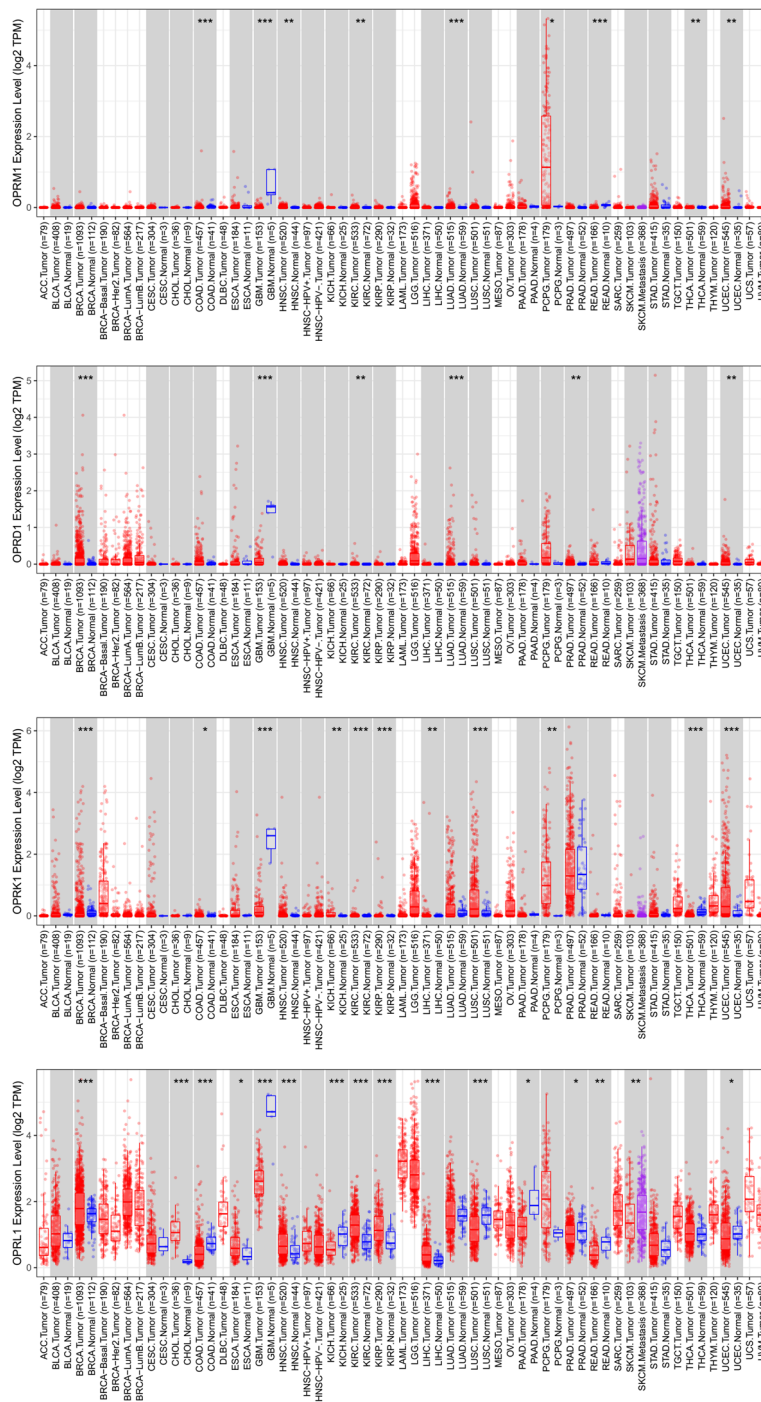
Fig. 2 The expression levels of four ORs between tumor and normal samples across TCGA cancer dataset. Pan-cancer analysis revealed that *OPRL1* exhibited a significantly higher abundance than the other three ORs across different types of cancers. In addition, the expression levels of *OPRM1* and *OPRD1* were undetectable in multiple cancer subtypes. Collectively, these four ORs displayed significant downregulation or upregulation across various types of cancers. All data was shown as mean ± SD, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. TCGA, the cancer genome atlas; SD, standard deviation
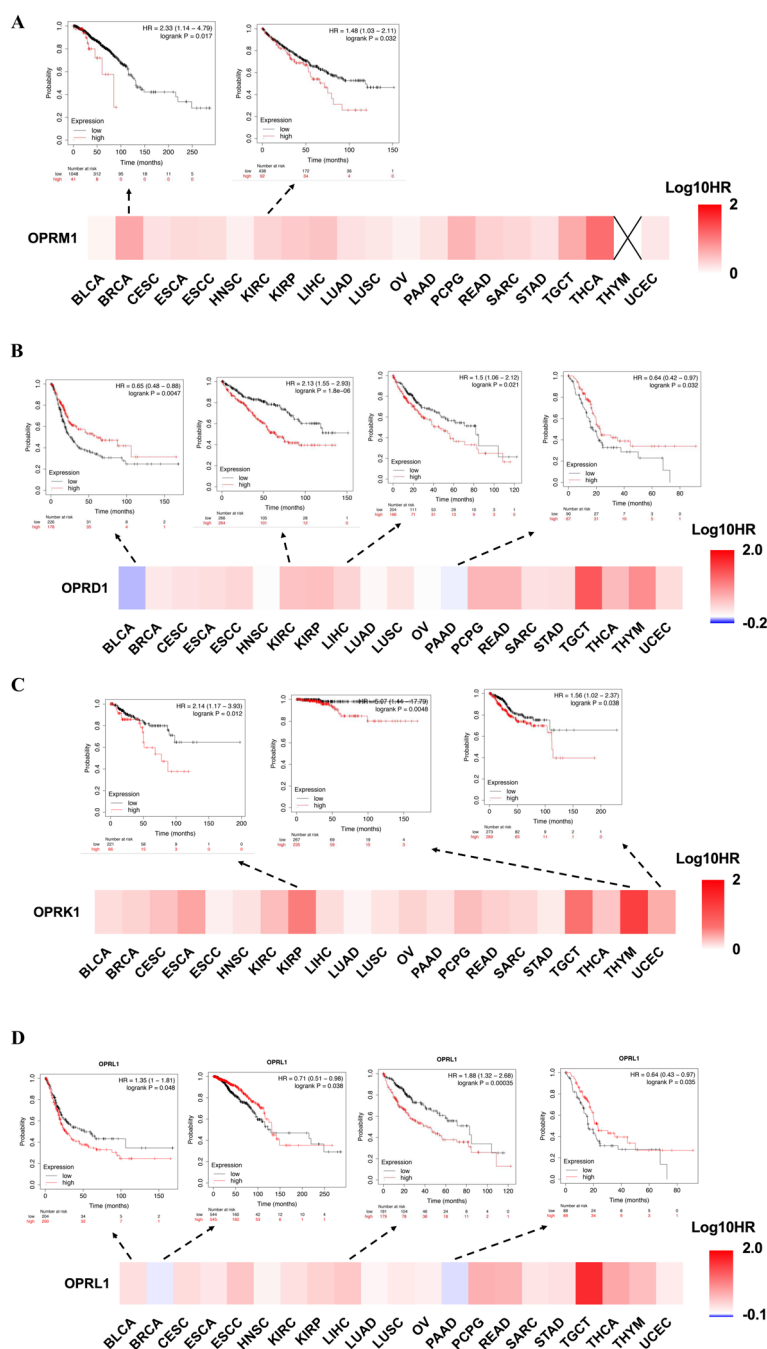
Fig. 3 The OS analyses of four ORs across TCGA cancer dataset. **A**. The OS analyses of *OPRM1* across TCGA cancer dataset. **B**. The OS analyses of *OPRD1* across TCGA cancer dataset. **C**. The OS analyses of *OPRK1* across TCGA cancer dataset. **D**. The OS analyses of *OPRL1* across TCGA cancer dataset. OS, overall survival; HR, hazard ratio

amplifications were found in *OPRK1* and *OPRL1* (Supplementary Fig. 2A). Additionally, the gene mutations of the four ORs were relatively low in cancers, ranging from 0 to 6.4% (Supplementary Fig. 2B).

3.3 Survival analysis of four ORs in the pan-cancer

Based on survival analyses, higher expression levels of *OPRM1* were associated with worse prognosis in BRCA and KIRC, as demonstrated by Figure 3A. Conversely, for
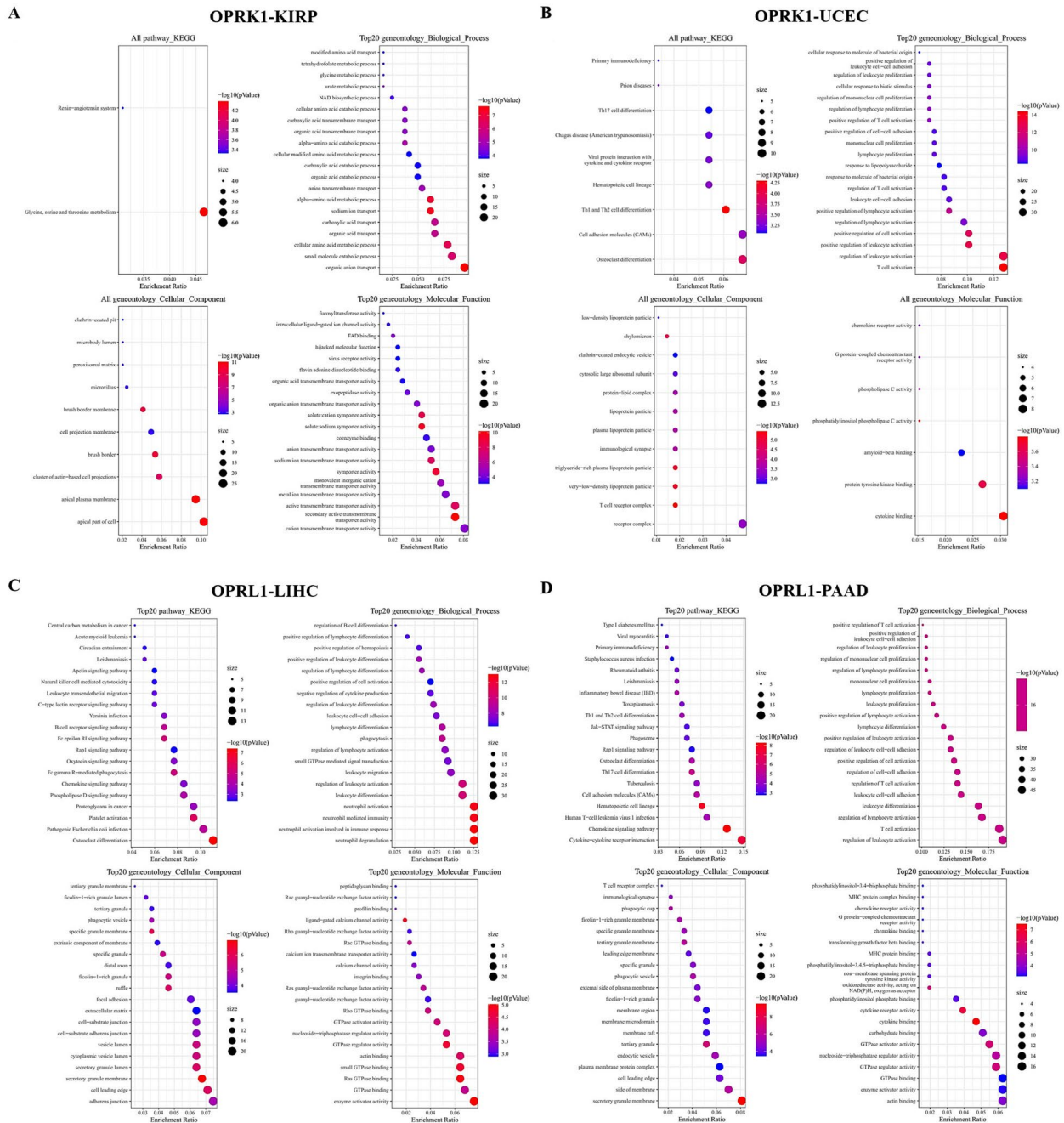
Fig. 4 Functional enrichment analyses and potential mechanism prediction of *OPRK1* and *OPRL1* in multiple cancers. **A**. Top enriched pathways of KEGG and GO in KIRP for *OPRK1*. Metabolic pathways such as amino acid metabolism and glycine metabolism were significantly enriched in KIRP. **B**. Top enriched pathways of KEGG and GO in UCEC for *OPRK1*. Immune-related pathways were dramatically enriched in UCEC. **C**. Analysis of LIHC revealed that *OPRL1* was associated with top enriched pathways in KEGG and GO analyses, with immune-related pathways exhibiting significant enrichment. **D**. Top enriched pathways of KEGG and GO in PAAD for *OPRL1*. Immune-related pathways were dramatically enriched in PAAD. KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology; KIRP, Kidney renal papillary cell carcinoma; UCEC, Uterine Corpus Endometrial Carcinoma; LIHC, liver hepatocellular carcinoma; PAAD, pancreatic adenocarcinoma
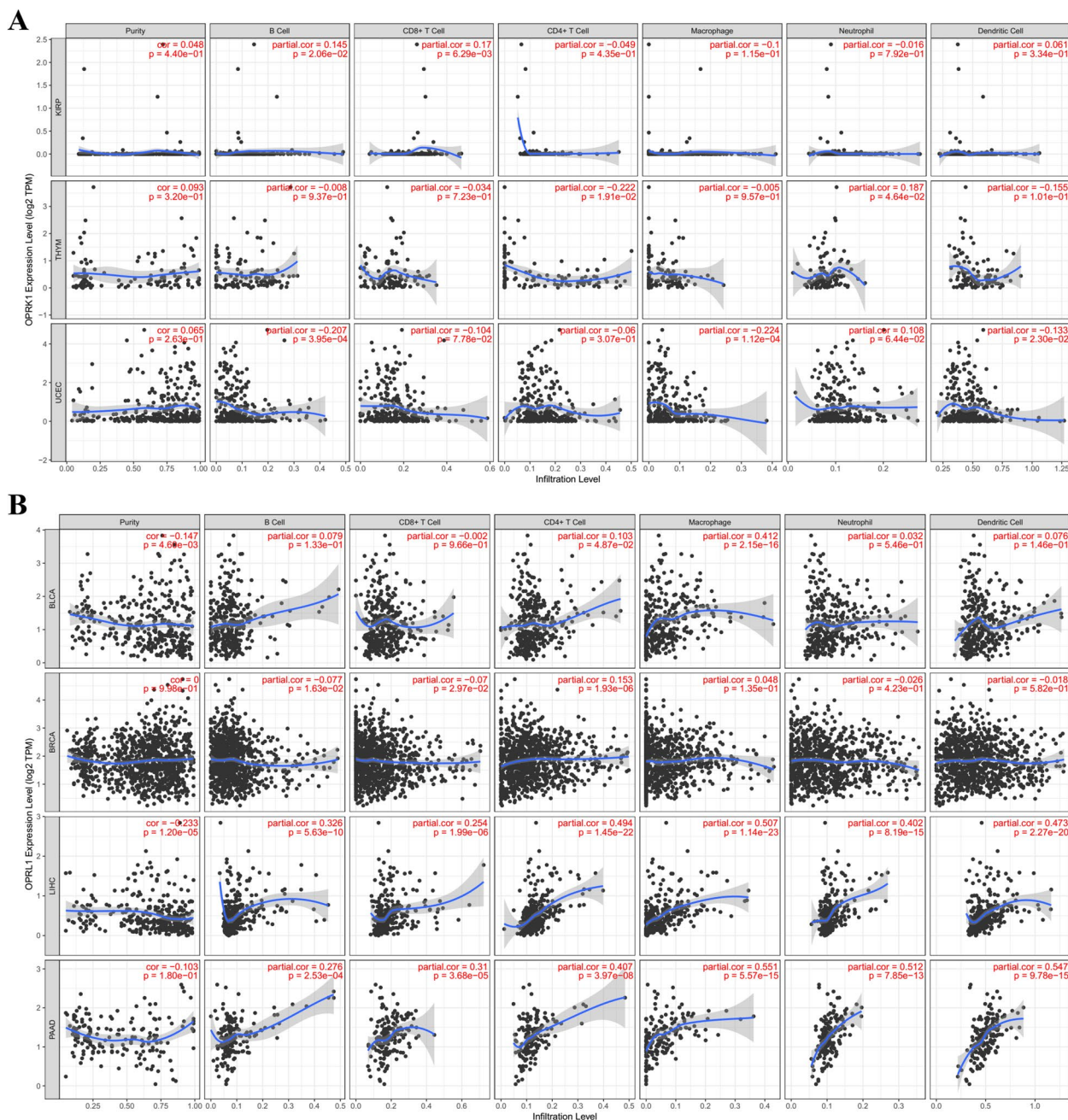
Fig. 5 Relationships among *OPRK1* and *OPRL1* expression levels and immune cell infiltration levels. **A**. Scatter plots between *OPRK1* expression levels and immune cell infiltration levels. **B**. Scatter plots between *OPRL1* expression levels and immune cell infiltration levels. Regression analyses suggested that higher expression levels of *OPRL1* correlated with higher infiltration levels of immune cell in four kinds of cancers especially in LIHC and PAAD

(See figure on next page.)

Fig. 6 mRNA expression levels of ORs and the effects of NOPR agonist on immune and HCC cell lines. A. mRNA expression levels of ORs in immune cell lines. B. Effect of NOPR agonist on the growth of THP-1. C. mRNA expression levels of ORs in normal hepatic cell line (LO-2) and HCC cell lines. D. Effects of NOPR agonist on the proliferation of BLCA, BRCA, LIHC and PAAD. E. Effects of NOPR agonist on the migration and invasion of BLCA, BRCA, LIHC and PAAD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ns, not significant. NOPR, nociception/orphanin; FQ, peptide receptor; BRCA, breast cancer; BLCA, bladder cancer
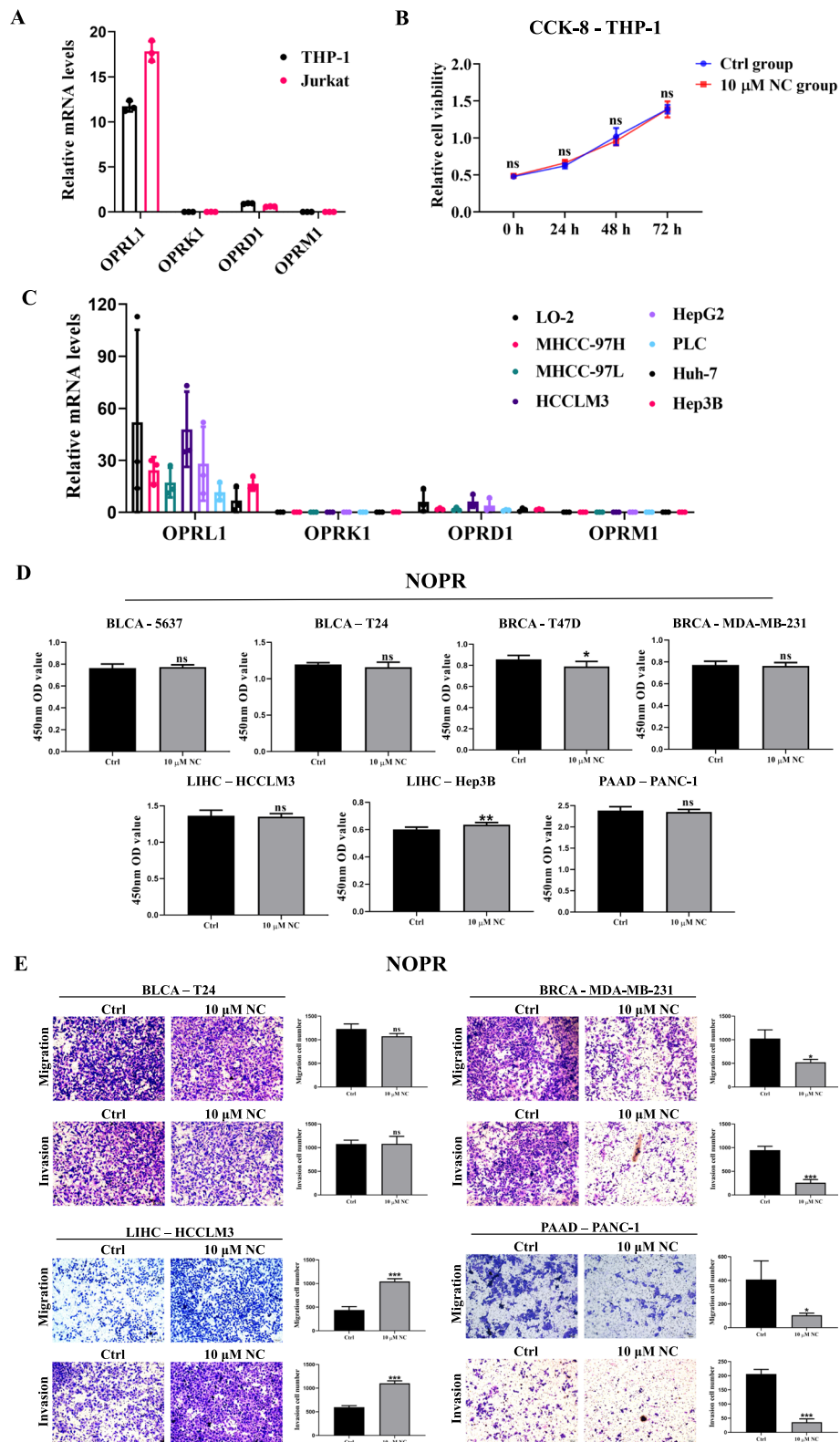
Fig. 6 (See legend on previous page.)

OPRD1, higher expression levels were associated with worse prognosis in KIRC and LIHC, but better prognosis in BLCA and PAAD, as shown in Figure 3B. In the case of *OPRK1*, higher expression levels were linked to worse prognosis in KIRP, thymoma, and UCEC, as displayed in Figure 3C. Finally, for *OPRL1*, higher expression levels were associated with worse prognosis in BLCA and LIHC, but better prognosis in BRCA and PAAD (Figure 3D).

3.4 Functional enrichment analyses of ORs
Co-expression genes of ORs in specific cancers were selected for functional enrichment analyses. For certain cancer types, we observed inadequate potential pathways enriched, with less than 10 for KEGG or GO-BP/GO-CC/GO-MF, and therefore did not present these results. Top enriched pathways of KEGG and GO in KIRP and UCEC for *OPRK1* were shown in Fig. 4A–B. Metabolic pathways such as amino acid metabolism and glycine metabolism were significantly enriched in KIRP (Fig. 4A) while immune-related pathways, such as T cell activation and regulation of leukocyte activation, were dramatically enriched in UCEC (Fig. 4B).

Interestingly, immune-related pathways were significantly enriched both in LIHC and PAAD for *OPRL1*, which strongly suggested that *OPRL1* might play a key role in immune response and tumor immune microenvironment regulation. Functional enrichment analyses for *OPRD1* in LIHC and PAAD revealed connections with cell cycle and proliferation-related pathways, as well as hormone and synapse-related pathways (Supplementary Fig. 3).

3.5 Correlations between tumor immune cell infiltration and ORs
Results revealed that *OPRL1* had the highest correlations with immune cell infiltration in comparison to the other three ORs (Fig. 5 & Supplementary Fig. 4). Notably, there was a significant positive correlation between the expression level of *OPRL1* and multiple types of immune cells infiltrating LIHC and PAAD, as depicted in Fig. 5B. Conversely, weak relationships were observed between immune cell infiltration and *OPRM1*/*OPRD1*, as presented in Supplementary Fig. 4. These outcomes provide additional evidence suggesting that *OPRL1* may play a role in tumor immune response.

3.6 Expression levels of OPRL1 in LIHC cell lines and immune cells
Next, the mRNA expression levels of the four different ORs were analyzed in a monocyte cell line (THP-1) and a T cell line (Jurkat), respectively, to validate the bioinformatic analysis. As shown in Fig. 6A, the expression levels of *OPRL1* were obviously higher than all other three ORs in both the two immune cell lines. Notably, Nociceptin, an agonist of NOPR, did not influence the growth of monocyte cell, as detected by viability assay (Fig. 6B). In a subsequent analysis of the mRNA expression levels of *OPRL1* in seven LIHC cell lines and one normal hepatic cell line (LO-2), the results were also consistent with bioinformatic analyses (Fig. 2), which revealed that *OPRL1* was highly expressed in all hepatic cancer and normal cell lines (Fig. 6C). In sharp contrast, the expression level of *OPRD1* was modest, whereas the levels of *OPRK1* and *OPRM1* were almost undetectable in these cell lines.

3.7 Effects of ORs agonists on the proliferation, migration and invasion of cancers in *vitro*
Experiments in vitro were performed to explore effects of ORs agonists on the proliferation, migration and invasion of specific types of cancers selected by the survival analyses (Fig. 3). CCK-8 assays demonstrated that the MOR agonist had negligible effects on cell proliferation in BRCA and KIRC (Supplementary Fig. 5A). DOR agonist could inhibit the growth of BLCA while promote the growth of KIRC and LIHC (Hep3B) (Supplementary Fig. 5B). KOR agonist significantly promoted the proliferation of KIRP and UCEC (Supplementary Fig. 5C). Conversely, the NOPR agonist had no significant effects on cell growth in BLCA, BRCA (MDA-MBA231), LIHC (HCCLM3) and PAAD (Fig. 6D). A slight inhibition was observed in T47D, while promotion was noted in Hep3B (Fig. 6D).

Transwell migration and invasion assays revealed that the MOR agonist could significantly increase the migration of BRCA and KIRC, as well as the invasion of BRCA (Supplementary Fig. 6A). The DOR agonist was observed to increase the migration and invasion of KIRC and LIHC, while opposite effects were observed in BLCA and PAAD (Supplementary Fig. 6B). Additionally, the KOR agonist significantly stimulated the invasion of KIRP and UCEC, as well as the migration of KIRP (Supplementary Fig. 6C). Moreover, the NOPR agonist was found to increase the migration and invasion of LIHC, while opposite effects were observed in BRCA and PAAD (Fig. 6E).

4 Discussion
This study is a general analysis of the four opioid receptors in pan-cancers, providing a prognostic and initial analysis, and is accompanied by preliminary in vitro experiments for validation. Based on the analysis of ORs expression levels in different normal tissues, we specifically found that *OPRL1* was highly expressed in monocytes. Analysis of ORs expression in immune cells also

revealed that *OPRL1* was significantly higher than the other three ORs. These results indicated that *OPRL1* may have an essential role in monocytes. According to the pan-cancer analysis, the highest abundance of *OPRL1* was found and more arm-level gain and high amplication were found in *OPRK1* and *OPRL1*. It is noteworthy that the expression level changes between normal and tumor that the TPMs are low and that the changes may be hitch-hikers driven by rare tumor genotypes, rather than reflective of functionally consequential expression.

Prognostic analysis pointed that the ORs acted as protective factors and risk factors in different cancer types. Functional enrichment analysis and tumor immune cell infiltration correlation studies showed that immune-related pathways were significantly enriched for *OPRK1* in UCEC, as well as for *OPRL1* in LIHC and PAAD. In both tumors, *OPRL1* expression levels were strongly and positively correlated with multiple immune cell infiltrations. In vitro cellular experiments using ORs agonists were validated and all results were consistent with the findings of the bioinformatic analysis. Together, these results demonstrated a general map of the relationship between four ORs and tumors. It also highlights the possible role of *OPRL1*, which has been less frequently mentioned before, in the regulation of the immune response and TIM.

Many studies over the past decade have found associations between ORs expression levels and the outcomes in specific cancer types [23, 43], which is consistent with the findings of our study. The implication is that at the molecular level, opioid agonism at the receptor has downstream effects that impact the oncological endpoint. To date, as MOR agonists, morphine and fentanyl are the leading analgesics in clinical treatment of moderate and severe pain, MOR has greater clinical relevance [44]. Thus, there are relatively more research on MOR and cancer [21, 45, 46]. DOR has been relatively well studied in breast cancer, with reports of promoting tumor metastasis and angiogenesis through epithelial-mesenchymal transition and activation of HIF-1α [47, 48]. Previous researches have revealed that KOR was increased in a variety of solid tumors, including liver cancer and esophageal squamous cell carcinoma, and the KOR expression is linked to cancer progression and bad prognosis [49, 50]. However, further studies are required to confirm the underlying mechanisms. In our study, the expression of *OPRM1* and *OPRD1* are very low in many cancers, and more arm-level deletion were found. For *OPRK1*, we reported the immune-related pathways were enriched in UCEC. In the endometrium, KOR is the most common opioid binding site [51]. This may provide a strong hint for subsequent research. There are few existing articles

that synthesize multiple ORs for side-by-side comparisons or outline their roles comprehensively. Hashimi *et.al* detected transcripts for four ORs in whole human blood from healthy donors which contained full range of circulating immune cells. They found only NOPR mRNA, with no MOR, DOR or KOR in these samples [52]. This result complements our study and suggests a potential application of NOPR in immunity. Belltall et al. also explored the relationship between the expression of ORs (MOR, KOR, DOR) and oncologic outcomes across various solid tumors [53]. While they conducted a purely biochemical analysis, our study delved more deeply into the subject matter.

Current research on NOPR has primarily focused on neurology and psychiatry. Preclinical models have investigated the efficacy of NOPR agonists for anxiety, cough, substance abuse, migraine, pain (spinal and peripheral), and urinary incontinence, while NOPR antagonists have been studied for their potential in treating pain, depression, and motor symptoms in Parkinson's disease [54–56]. Another objective of research is to identify ligands and conduct analgesic studies to develop new anesthetic drugs with greater selectivity and safety. Regarding immunology research, NOPR mRNA has been identified in blood lymphocytes, monocytes, and granulocytes from healthy human donors using PCR or qPCR methods [52, 57], which is in line with our bioinformatic analysis results. However, few existing studies have examined the interaction between *OPRL1* and tumors. Szalay et al. found that nociception was elevated in 18 hepatocellular carcinoma patients with a ten-fold increase compared to other liver diseases in a study of plasma nociception levels between liver diseases and healthy controls [58]. Similar results were shown in another study of 113 patients with different types of advanced cancer [59].

Additionally, some studies have explored the mechanisms underlying these phenomena. After Kuzumaki et al. reported upregulation of *OPRL1* in Non-small cell lung cancer (NSCLC) for the first time [60], Wang and his colleagues found that NOPR is overexpressed in NSCLC and is inversely correlated with patients' postoperative survival, acting via the PI3K/Akt signaling pathway [61]. Studies on NOPR antagonist JTC-801 have shown inhibition of proliferation and metastasis in ovarian cancer cells [62], melanoma cells [63], and osteosarcoma cells [64] through the PI3K/AKT pathway. In our study, we discovered that *OPRL1* is not only highly expressed in tumors, but also closely related to tumor immunity, which has not been previously reported. Since *OPRL1* has the highest abundance in different cancers compared to other ORs, it may have greater clinical significance for

oncology patients, and it can guide the development of new drugs targeting NOPR.

The limitations of the present study merit attention. Firstly, validation of immune-related signaling pathways enriched by functional analysis requires in vivo or in vitro experiments. While some validation was performed using cell lines in our study, this offers a promising avenue for further exploration by scientists. Secondly, additional research is imperative to elucidate the mechanism and clinical significance of modulated OR levels in specific tumors. Our group is embarking on the next phase of comprehensive investigation, utilizing animal models and single-cell RNA sequencing to unravel the relevant regulatory factors and mechanisms of action. This will establish the groundwork for improved treatment options for oncology patients.

5 Conclusion

By performing a pan-cancer bioinformatic analysis and fundamental validations in vitro, our study investigated the expression heterogeneity, tumor immune characteristics and the prognosis effects of four ORs on patients with tumors. *OPRL1* exhibited the highest abundance across different types of cancers, while *OPRM1* and *OPRD1* were barely detectable in multiple cancer types. Notably, our findings highlight the significant clinical importance of *OPRK1* and *OPRL1*, which may play a crucial role in tumor immunity. Furthermore, *OPRL1* may play a vital role in monocytes and regulating the immune response and tumor-infiltrating macrophages. Consequently, targeting KOR and NOPR could represent a promising avenue for cancer treatment. Nonetheless, further studies are warranted to explore connections between *OPRL1*/*OPRK1* and tumor immune regulation.

Abbreviations

BLCA	Bladder cancer
BRCA	Breast cancer
CCK-8	Cell counting kit
DOR	δ Opioid receptor
GO	Gene ontology
HPA	Human Protein Atlas
KEGG	Kyoto Encyclopedia of Genes and Genomes
KIRC	Kidney renal clear cell carcinoma
KIRP	Kidney renal papillary cell carcinoma
KOR	κ Opioid receptor
LIHC	Liver hepatocellular carcinoma
MOR	μ Opioid receptor
NOPR	Nociception/orphanin FQ peptide receptor
OPRD1	Opioid receptor delta 1 gene
OPRK1	Opioid receptor kappa 1 gene
OPRL1	Opioid related nociceptin receptor 1 gene
OPRM1	Opioid receptor mu 1 gene
OR	Opioid receptor
OS	Overall survival
PAAD	Pancreatic adenocarcinoma
sCNA	Significant copy number alteration
TCGA	The Cancer Genome Atlas
TIM	Tumor immune microenvironment
TISCH	Tumor Immune Single-cell Hub
UCEC	Uterine corpus endometrial carcinoma

Supplementary Information

Additional file 1.

Additional file 2.

Additional file 3.

Additional file 4.

Additional file 5.

Additional file 6.

Additional file 7.

Authors' contributions
Conceptualization, W.Y., J.T. and X.W.; methodology, X.W.; software, X.W.; validation, X.W. and Y.T.; formal analysis, X.W. and C.Z.; writing- original draft preparation, X.W. and Y.T.; writing-review and editing, W.Y. and J.T.; supervision, W.Y. and J.T.; funding acquisition, W.Y. and J.T.

Availability of data and materials
All data are available under reasonable request.

Declarations

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Not applicable.

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All authors gave their consent for publication.

Competing interests
Prof. Weifeng Yu is a member of the Editorial Board in Anesthesiology and Perioperative Science and recuses himself from every editorial procedure of this submission including peer-review and academic decisions. All authors declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

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References

1. Scarborough BM, Smith CB. Optimal pain management for patients with cancer in the modern era. CA Cancer J Clin. 2018;68(3):182–96.
2. Zhou Q, Zhang Z, Long S, Li W, Wang B, Liang N. Opioids in cancer: The κ-opioid receptor (Review). Mol Med Rep. 2022;25(2):44.
3. Carli M, Donnini S, Pellegrini C, Coppi E, Bocci G. Opioid receptors beyond pain control: The role in cancer pathology and the debated importance of their pharmacological modulation. Pharmacol Res. 2020;159:104938.
4. Waldhoer M, Bartlett SE, Whistler JL. Opioid receptors. Annu Rev Biochem. 2004;73:953–90.
5. Kiguchi N, Ding H, Kishioka S, Ko MC. Nociceptin/Orphanin FQ Peptide Receptor-Related Ligands as Novel Analgesics. Curr Top Med Chem. 2020;20(31):2878–88.
6. Gach K, Szemraj J, Wyrebska A, Janecka A. The influence of opioids on matrix metalloproteinase-2 and -9 secretion and mRNA levels in MCF-7 breast cancer cell line. Mol Biol Rep. 2011;38(2):1231–6.
7. Ma Y, Ren Z, Ma S, Yan W, He M, Wang D, et al. Morphine enhances renal cell carcinoma aggressiveness through promotes survivin level. Ren Fail. 2017;39(1):258–64.
8. Lu H, Zhang H, Weng ML, Zhang J, Jiang N, Cata JP, et al. Morphine promotes tumorigenesis and cetuximab resistance via EGFR signaling activation in human colorectal cancer. J Cell Physiol. 2021;236(6):4445–54.
9. Ustun F, Durmus-Altun G, Altaner S, Tuncbilek N, Uzal C, Berkarda S. Evaluation of morphine effect on tumour angiogenesis in mouse breast tumour model. EATC Med Oncol. 2011;28(4):1264–72.
10. Gupta K, Kshirsagar S, Chang L, Schwartz R, Law PY, Yee D, et al. Morphine stimulates angiogenesis by activating proangiogenic and survival-promoting signaling and promotes breast tumor growth. Cancer Res. 2002;62(15):4491–8.
11. Vijayakumar J, Haddad T, Gupta K, Sauers J, Yee D. An open label phase II study of safety and clinical activity of naltrexone for treatment of hormone refractory metastatic breast cancer. Invest New Drugs. 2023;41(1):70–5.
12. Hasegawa T, Oguri T, Osawa T, Sawa T, Osaga S, Okuyama T, et al. Opioid Dose and Survival of Patients with Incurable Nonsmall Cell Lung Cancer: A Prospective Cohort Study. J Palliat Med. 2018;21(10):1436–41.
13. Guerrero Orriach JL, RaigonPonferrada A, MaloManso A, Herrera Imbroda B, Escalona Belmonte JJ, Ramirez Aliaga M, et al. Anesthesia in Combination with Propofol Increases Disease-Free Survival in Bladder Cancer Patients Who Undergo Radical Tumor Cystectomy as Compared to Inhalational Anesthetics and Opiate-Based Analgesia. Oncology. 2020;98(3):161–7.
14. Zylla D, Kuskowski MA, Gupta K, Gupta P. Association of opioid requirement and cancer pain with survival in advanced non-small cell lung cancer. Br J Anaesth. 2014;113(Suppl 1):i109–16.
15. Bimonte S, Barbieri A, Palma G, Arra C. The role of morphine in animal models of human cancer: does morphine promote or inhibit the tumor growth? Biomed Res Int. 2013;2013:258141.
16. Gong S, Ying L, Fan Y, Sun Z. Fentanyl Inhibits Lung Cancer Viability and Invasion via Upregulation of miR-331-3p and Repression of HDAC5. Onco Targets Ther. 2020;13:13131–41.
17. Li C, Qin Y, Zhong Y, Qin Y, Wei Y, Li L, et al. Fentanyl inhibits the progression of gastric cancer through the suppression of MMP-9 via the PI3K/Akt signaling pathway. Ann Transl Med. 2020;8(4):118.
18. Novy DM, Nelson DV, Koyyalagunta D, Cata JP, Gupta P, Gupta K. Pain, opioid therapy, and survival: a needed discussion. Pain. 2020;161(3):496–501.
19. Fichna J, Janecka A. Opioid peptides in cancer. Cancer Metastasis Rev. 2004;23(23–24):351–66.
20. Santoni A, Santoni M, Arcuri E. Chronic Cancer Pain: Opioids within tumor microenvironment affect neuroinflammation, tumor and pain evolution. Cancers (Basel). 2022;14(9):2253.
21. Singleton PA, Mirzapoiazova T, Hasina R, Salgia R, Moss J. Increased mu-opioid receptor expression in metastatic lung cancer. Br J Anaesth. 2014;113(Suppl 1):i103–8.
22. Li Y, Li G, Tao T, Kang X, Liu C, Zhang X, et al. The μ-opioid receptor (MOR) promotes tumor initiation in hepatocellular carcinoma. Cancer Lett. 2019;453:1–9.
23. Zylla D, Gourley BL, Vang D, Jackson S, Boatman S, Lindgren B, et al. Opioid requirement, opioid receptor expression, and clinical outcomes in patients with advanced prostate cancer. Cancer. 2013;119(23):4103–10.
24. Maman S, Witz IP. A history of exploring cancer in context. Nat Rev Cancer. 2018;18(6):359–76.
25. Zhang H, Zhou D, Gu J, Qu M, Guo K, Chen W, et al. Targeting the mu-Opioid Receptor for Cancer Treatment. Curr Oncol Rep. 2021;23(10):111.
26. Gavériaux-Ruff C, Matthes HW, Peluso J, Kieffer BL. Abolition of morphine-immunosuppression in mice lacking the mu-opioid receptor gene. Proc Natl Acad Sci USA. 1998;95(11):6326–30.
27. Houshyar H, Cooper ZD, Woods JH. Paradoxical effects of chronic morphine treatment on the temperature and pituitary-adrenal responses to acute restraint stress: a chronic stress paradigm. J Neuroendocrinol. 2001;13(10):862–74.
28. Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, et al. Proteomics. Tissue-based map of the human proteome. Science. 2015;347(6220):1260419.
29. Uhlen M, Karlsson MJ, Zhong W, Tebani A, Pou C, Mikes J, et al. A genome-wide transcriptomic analysis of protein-coding genes in human blood cells. Science. 2019;366(6472):eaax9198.
30. Monaco G, Lee B, Xu W, Mustafah S, Hwang YY, Carré C, et al. RNA-Seq Signatures Normalized by mRNA Abundance Allow Absolute Deconvolution of Human Immune Cell Types. Cell Rep. 2019;26(6):1627–40.e7.
31. Ratnapriya R, Sosina OA, Starostik MR, Kwicklis M, Kapphahn RJ, Fritsche LG, et al. Retinal transcriptome and eQTL analyses identify genes associated with age-related macular degeneration. Nat Genet. 2019;51(4):606–10.
32. Li T, Fu J, Zeng Z, Cohen D, Li J, Chen Q, et al. TIMER2.0 for analysis of tumor-infiltrating immune cells. Nucleic Acids Res. 2020;48(W1):W509–14.
33. Fonseca-Montaño MA, Blancas S, Herrera-Montalvo LA, Hidalgo-Miranda A. Cancer Genomics. Arch Med Res. 2022;53(8):723–31.
34. Zarrei M, MacDonald JR, Merico D, Scherer SW. A copy number variation map of the human genome. Nat Rev Genet. 2015;16(3):172–83.
35. Lánczky A, Győrffy B. Web-Based Survival Analysis Tool Tailored for Medical Research (KMplot): Development and Implementation. J Med Internet Res. 2021;23(7):e27633.
36. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal. 2013;6(269):pl1.
37. Jenkins RW, Aref AR, Lizotte PH, Ivanova E, Stinson S, Zhou CW, et al. Ex Vivo Profiling of PD-1 Blockade Using Organotypic Tumor Spheroids. Cancer Discov. 2018;8(2):196–215.
38. Newman A, Liu C, Green M, Gentles A, Feng W, Xu Y, et al. Robust enumeration of cell subsets from tissue expression profiles. Nat Methods. 2015;12(5):453–7.
39. Sun D, Wang J, Han Y, Dong X, Ge J, Zheng R, et al. TISCH: a comprehensive web resource enabling interactive single-cell transcriptome visualization of tumor microenvironment. Nucleic Acids Res. 2021;49(D1):D1420–30.
40. Lahti RA, Mickelson MM, McCall JM, Von Voigtlander PF. U-69593 a highly selective ligand for the opioid kappa receptor. Eur J Pharmacol. 1985;109(2):281–4.
41. Schenk S, Partridge B, Shippenberg TS. Reinstatement of extinguished drug-taking behavior in rats: effect of the kappa-opioid receptor agonist, U69593. Psychopharmacology. 2000;151(1):85–90.
42. Wang X, Zhang S, Jin D, Luo J, Shi Y, Zhang Y, et al. μ-opioid receptor agonist facilitates circulating tumor cell formation in bladder cancer via the MOR/AKT/Slug pathway: a comprehensive study including randomized controlled trial. Cancer Commun (Lond). 2023;43(3):365–86.
43. Connolly C, Madden SF, Buggy DJ, Gallagher HC. Expression of anaesthetic and analgesic drug target genes in excised breast tumour tissue: Association with clinical disease recurrence or metastasis. PLoS ONE. 2017;12(5):e0177105.
44. Wolff RF, Aune D, Truyers C, Hernandez AV, Misso K, Riemsma R, et al. Systematic review of efficacy and safety of buprenorphine versus fentanyl or morphine in patients with chronic moderate to severe pain. Curr Med Res Opin. 2012;28(5):833–45.
45. Singleton PA, Moss J, Karp DD, Atkins JT, Janku F. The mu opioid receptor: A new target for cancer therapy? Cancer. 2015;121(16):2681–8.
46. Cieslinska A, Sienkiewicz-Szlapka E, Kostyra E, Fiedorowicz E, Snarska J, Wronski K, et al. μ-Opioid receptor gene (OPRM1) polymorphism in patients with breast cancer. Tumour Biol. 2015;36(6):4655–60.

47. Tripolt S, Neubauer HA, Knab VM, Elmer DP, Aberger F, Moriggl R, et al. Opioids drive breast cancer metastasis through the delta-opioid receptor and oncogenic STAT3. Neoplasia. 2021;23(2):270–9.

48. Schoos A, Gabriel C, Knab VM, Fux DA. Activation of HIF-1alpha by delta-Opioid Receptors Induces COX-2 Expression in Breast Cancer Cells and Leads to Paracrine Activation of Vascular Endothelial Cells. J Pharmacol Exp Ther. 2019;370(3):480–9.

49. Chen D, Chen Y, Yan Y, Pan J, Xing W, Li Q, et al. Down-regulation of the tumour suppressor kappa-opioid receptor predicts poor prognosis in hepatocellular carcinoma patients. BMC Cancer. 2017;17(1):553.

50. Zhang YF, Xu QX, Liao LD, Xu XE, Wu JY, Shen J, et al. κ-Opioid receptor in the nucleus is a novel prognostic factor of esophageal squamous cell carcinoma. Hum Pathol. 2013;44(9):1756–65.

51. Hatzoglou A, Gravanis A, Margioris AN, Zoumakis E, Castanas E. Identification and characterization of opioid-binding sites present in the Ishikawa human endometrial adenocarcinoma cell line. J Clin Endocrinol Metab. 1995;80(2):418–23.

52. Al-Hashimi M, McDonald J, Thompson JP, Lambert DG. Evidence for nociceptin/orphanin FQ (NOP) but not micro (MOP), delta (DOP) or kappa (KOP) opioid receptor mRNA in whole human blood. Br J Anaesth. 2016;116(3):423–9.

53. Belltall A, Zuniga-Trejos S, Garrido-Cano I, Eroles P, Argente-Navarro MP, Buggy DJ, et al. Solid Tumor Opioid Receptor Expression and Oncologic Outcomes: Analysis of the Cancer Genome Atlas and Genotype Tissue Expression Project. Front Oncol. 2022;12:801411.

54. Zaveri NT. Nociceptin Opioid Receptor (NOP) as a Therapeutic Target: Progress in Translation from Preclinical Research to Clinical Utility. J Med Chem. 2016;59(15):7011–28.

55. Toll L, Cippitelli A, Ozawa A. The NOP Receptor System in Neurological and Psychiatric Disorders: Discrepancies, Peculiarities and Clinical Progress in Developing Targeted Therapies. CNS Drugs. 2021;35(6):591–607.

56. Targowska-Duda KM, Ozawa A, Bertels Z, Cippitelli A, Marcus JL, Mielke-Maday HK, et al. NOP receptor agonist attenuates nitroglycerin-induced migraine-like symptoms in mice. Neuropharmacology. 2020;170:108029.

57. Machelska H, Celik MO. Opioid Receptors in Immune and Glial Cells-Implications for Pain Control. Front Immunol. 2020;11:300.

58. Szalay F, Hantos MB, Horvath A, Lakatos PL, Folhoffer A, Dunkel K, et al. Increased nociceptin/orphanin FQ plasma levels in hepatocellular carcinoma. World J Gastroenterol. 2004;10(11):42–5.

59. Stamer UM, Book M, Comos C, Zhang L, Nauck F, Stuber F. Expression of the nociceptin precursor and nociceptin receptor is modulated in cancer and septic patients. Br J Anaesth. 2011;106(4):566–72.

60. Kuzumaki N, Suzuki A, Narita M, Hosoya T, Nagasawa A, Imai S, et al. Multiple analyses of G-protein coupled receptor (GPCR) expression in the development of gefitinib-resistance in transforming non-small-cell lung cancer. PLoS ONE. 2012;7(10):e44368.

61. Wang K, Zheng Y, Yang Y, Wang J, Li B, Wei F, et al. Nociceptin Receptor Is Overexpressed in Non-small Cell Lung Cancer and Predicts Poor Prognosis. Front Oncol. 2019;9:235.

62. Li JX, Bi YP, Wang J, Yang X, Tian YF, Sun ZF. JTC-801 inhibits the proliferation and metastasis of ovarian cancer cell SKOV3 through inhibition of the PI3K - AKT signaling pathway. Pharmazie. 2018;73(5):283–7.

63. Li J, Huang F. JTC-801 Suppresses Melanoma Cells Growth through the PI3K-Akt-mTOR Signaling Pathways. Med Sci (Paris). 2018;34 Focus issue F1:8–14.

64. Zheng CJ, Yang LL, Liu J, Zhong L. JTC-801 exerts anti-proliferative effects in human osteosarcoma cells by inducing apoptosis. J Recept Signal Transduct Res. 2018;38(2):133–40.

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