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Original Article

Pan-cancer analysis of the oncogenic role of the core osteosarcoma gene VCAN in human tumors

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Abstract: Objective: Versican (VCAN), a member of the multifunctional glycoprotein family, is involved in various aspects of cancer progression. However, the role of VCAN in diverse cancers remains poorly defined. This research aimed to investigate the correlation between VCAN expression and the oncogenic role, as well as visualize its prognostic landscape in pan-cancer. Methods: Raw data in regard to VCAN expression in cancer patients were acquired from GEO GeneChip public database in NCBI. Besides, we selected microarray data GSE16088 for analysis. We retrieved the genes associated with osteosarcoma (OS) from the OMIM database and identified their intersection with the core module. VCAN was suggested to be a potential marker gene for OS. Subsequently, we conducted Gene Set Enrichment Analysis (GSEA) to explore gene functional enrichment. Moreover, we performed pan-cancer analysis on VCAN to gain a comprehensive understanding of its implications across various cancer types. Results: The VCAN expression in the tumor tissue was higher than that in normal tissue. Elevated expression of VCAN was associated with high the tumor stage and poor long-term survival. There was a significant positive correlation between VCAN and cancer fibroblasts in all pan cancers. Moreover, FBN1 was the intersection gene of VCAN-related genes and linker genes. ANTXR1, COL5A2, CSGALNACT2, and SPARC were the target genes of VCAN genes. GSEA analysis showed that VCAN was mainly enriched in the extracellular matrix (ECM) signaling pathway. Conclusion: VCAN can be used as a marker molecule for the early diagnosis of OS and holds significance as a molecule in cases of OS with distant metastasis. The ECM signaling pathway may be a core pathway in OS development and distant metastasis. These findings shed new light on therapeutics of cancers.

Keywords: Osteosarcoma, pan cancer, VCAN, ECM signaling pathway, transfer

Introduction

Cancer can be viewed as an uncontrolled, unrestrained, genetically chaotic disease. It has been found that the local microenvironment of cancer cells plays an important role in tumor development. This microenvironment encompasses significant constituents, among which the extracellular matrix (ECM) holds particular prominence [1]. ECM is a non-cellular three-dimensional (3D) macromolecular network of proteoglycans/glycosaminoglycans, collagen, elastin, fibronectin, laminin, and other glycoproteins, with unique physical, biochemical, and biomechanical properties that provide necessary structural and biochemical support for cellular components [2]. ECM is involved in a variety of biological processes in tumors, including osteosarcomas, such as the transmission of extracellular signaling cascades to the intracellular compartment, and the regulation of cell communication, migration, adhesion, proliferation, and differentiation [3].

Versican (VCAN), a large aggregating chondroitin sulfate proteoglycan belonging to the lexicon family, is an important ECM component, and is closely associated with tumorigenesis, such as cell adhesion, proliferation, migration, and angiogenesis. These functions are attributed to the central glycosaminoglycan-binding region of VCAN and the N-(G1) and C-(G3) terminal globular domains that act in concert with many ECMs and cell surface structural components [4, 5]. Previous studies have shown that VCAN deposits in the tumor mesenchyme and interacts with

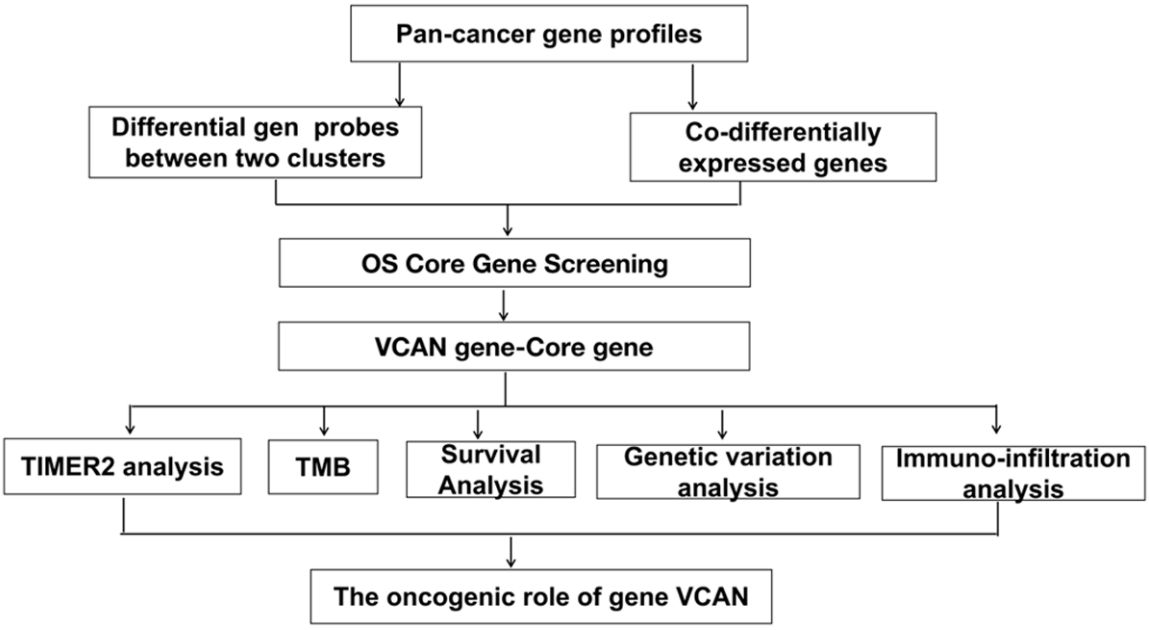

Figure 1. The flow chart for this study.

other ECM components to play an important role in the progression and regression of various inflammatory and tumorigenic diseases [6, 7]. Upregulation of VCAN was observed in a variety of cancers, such as gastric cancer, bladder cancer, breast cancer, colon cancer, and esophageal cancer [8-11]. Currently, VCAN has received considerable attention as both a promising biomarker and a vital mediator of human cancers, but the relevance of VCAN function with the tumorigenesis is still unknown.

Therefore, we used gene expression omnibus (GEO) and OMIM databases to obtain the osteosarcoma (OS)-related Hub gene-VCAN, screened and confirmed the abnormal expression of VCAN in OS for the first time, performed pan-cancer analysis of VCAN to observe the expression variation of VCAN among pan-cancers, and analyzed the effect of VCAN and ECM-related action pathways on the development of OS and other cancers. The objective is to understand the connection between OS and other cancers as well as to provide new targets for the diagnosis and treatment of OS.

Materials and methods

General materials

Dataset GSE16088 gene profile microarray data were acquired from the GEO database of the National Center for Biotechnology Information. Furthermore, GSE16088 is associated with pan cancer. The data were published by Benisch et al. [5] in 2012 and annotated by HGU133 Plus 2.0 (Affymetrix, Inc., USA) on a GPL96 microarray platform. The data contained 6 normal tissues and 14 OS tissues. This study was designed and performed according to the flow chart presenting in **Figure 1**.

Expression analysis of differential genes

In this study, we used the R language Limma package to perform differential expression analysis on the GSE16088 dataset and set the test statistic $P<0.05$ and the log absolute value of fold change (FC) $|\log FC|>2.5$ as the conditions to screen the differentially expressed mRNAs (DEmRNAs) in advanced OS articular cartilage and normal tissues.

Analysis of core modules

The differential genes were analyzed by STRING (a search tool for the retrieval of interacting genes/proteins) 11.0 online tool. The calculation results of STRING were imported into Cytoscape 3.9.1 software, and the plug-in MCODE was used to find the core modules associated with OS in the protein interaction network.

OS-related core gene screening

The keyword “Osteosarcoma” was searched in OMIM (https://omim.org/) to find genes associ-

ated with OS based on the existing literature. These genes were then cross-referenced with key protein molecules obtained from MCODE analysis using Venn diagrams.

Gene set enrichment analysis (GSEA) of pathways

In order to explore the biological signaling pathways, GSEA was performed in the high expression and the low expression groups, respectively, using the median VCAN expression level as the cut-off value. The top five terms of KEGG and HALLMARK analyses were exhibited. KEGG pathways with significant enrichment results were demonstrated based on Net enrichment score (NES), gene ratio, and *P* value. Gene sets with |NES|>1, NOM p<0.05, and FDR q<0.25 were considered to be enrichment significant.

Gene expression analysis

The core genes (VCAN) screened above were entered into the "Differ Exp" module of TIMER (Tumor IMmune Estimation Resource), a website (https://cistrome.shinyapps.io/timer/), to observe the differences in VCAN expression between tumors and adjacent normal tissues or specific tumor subtypes in the TCGA project. For certain tumors without normal or highly limited normal tissues (e.g., adrenocortical carcinoma (ACC), ovarian cancer, and diffuse large B-cell lymphoma (DLBC)), box plots of the expression differences between these tumors and the corresponding normal tissues were obtained using the "Expression Analysis - Box Plot" module of GEPIA2 (http://gepia2.cancer-pku.cn/#analysis) and the GTEx (genotype-tissue expression) database (P<0.05, |Log2FC| Cut). The dataset was evaluated according to clinical parameters (gender, cancer stage, metastasis), and values per million transcripts were calculated, expressed as Log2(TPM+1). Subsequently, box or violin plots of VCAN expression levels in different cancer patients in the TCGA database were plotted. In addition, the "Stage Plot" module of HEPIA2 was used to obtain violin maps of VCAN expression in all TCGA tumors at different pathological stages (stage I, II, III and IV).

The UALCAN database (http://ualcan.path.uab.edu/) is an interactive web resource for analyzing cancer omics data. This database enabled us to perform protein expression analysis on Clinical Proteomic Tumor Analysis Consortium, where we explored the expression levels of total or phosphoproteins of VCAN between primary tumors and normal tissues.

Survival prognosis analysis

We used the "Survival Analysis" module of GEPIA2 to obtain overall survival and disease-free survival data for VCAN in all TCGA tumors. Cutoff-High =50 and Cutoff-Low =50 were used as the expression threshold for splitting high and low expression cohorts. The log-rank test was used for hypothesis testing, and the survival plot was also obtained by the "Survival Analysis" module of GEPIA2.

Genetic variation analysis

Using the cBioPortal website (https://www.cbioportal.org/), we selected "TCGA Pan-Cancer Atlas Studies" and input "VCAN" to search for gene variant characteristics. The results of alteration frequency, mutation type, and copy number alteration for all TCGA tumors were observed in the "Cancer Types Summary" module. VCAN mutation site information can be displayed in protein structure schematics or 3D structures via the "Mutations" module. We also used the "Comparison/Survival" module to obtain data on overall, disease-free, progression-free, and disease-free survival differences in TCGA cancer cases with altered VCAN genes. Kaplan-Meier plots with log-rank *p*-values were also generated.

Immuno-infiltration analysis

The "Immune" module of the TIMER2 website was used to explore the association between VCAN expression and immune infiltration in all TCGA tumors. This involved selecting immune cells that are associated with cancer-associated fibroblasts. Our analysis incorporated various algorithms including TIMER, CIBERSORT, CIBERSORT-ABS, QUANTISEQ, XCELL, MCPCOUNTER, and EP-IC for immune infiltration estimation. *P*-values and partial correlation (COR) values were obtained by purity-adjusted Spearman's rank correlation test. Data are presented as heat and scatter plots.

Enrichment analysis of VCAN-related genes

By using the STRING database, a query was conducted using the protein name "VCAN" and species "Homo sapiens". The parameters were

set as follows: ① Interaction score: 0.150; ② The maximum number of displayed interaction objects was limited to 50, with interaction sourced from experiments. This resulted in the identification of a final set of 42 reciprocal proteins.

Using the "Similar Genes Detection" module of GEPIA2, the top 100 VCAN-related target genes were obtained from all TCGA tumor and normal tissue datasets, and the genes with high correlation were selected for paired gene Pearson correlation analysis with VCAN genes. The scatter plots are log2TPM, giving *P*-values, and correlation coefficients R. The heat map of the selected genes was then plotted, containing the R and *P* values. To refine the gene selection, the initial set of over 100 genes were analyzed with 42 genes that could interact with VCAN using the interactive Venn diagram viewer, Jvenn, so as to compare genes binding to VCAN with those interacting with it.

In addition, we combined the two data sets for gene ontology (GO)/Kyoto encyclopedia of gene sand genomes (KEGG) pathway analysis and visualized the analysis.

Statistical analysis

Gene expression data from GEO GeneChip public database in NCBI were analyzed using Student's t-test. The correlation analysis was evaluated in the TIMER database using Spearman's correlation analysis. The correlations between VCAN expression and abundance scores of immune cells were evaluated by Spearman's correlation. All analyses were performed with the R software (version 3.5.1, www.r-project.org) loaded with R packages (ggplot2, circlize, clusterProfiler, DOSE and enrichplot) to visualize the results. Results with $P<0.05$ were considered as statistically significant.

Results

Acquisition of DEmRNAs

In this study, GSE16088 was analyzed using the R language limma package, and most of the gene expressions of the 20 samples in the database remained largely consistent, indicating that the data are suitable for the next step of the analysis. See **Figure 2A**.

Further analysis of the GSE16088 dataset yielded a total of 708 DEmRNAs. These results were visually presented through volcano plots, wherein red dots signify up-regulated genes and blue dots signify down-regulated genes. See **Figure 2B**.

Modular analysis of co-differentially expressed genes

The 708 DEmRNAs were analyzed using the STRING online tool and the minimum interaction score was set at 0.4. The calculation results of STRING were imported into Cytoscape 3.9.1 software, and the protein interaction network was analyzed with the MCODE plug-in to obtain the DEmRNAs core module. See **Figure 2C**.

OS core gene screening

A systematic search within the OMIM online database for human genes and genetic disorders yielded a compilation of 508 genes associated with OS that have been reported in the literature. This gene list was then compared with 33 key protein molecules obtained from MCODE analysis using Venn diagrams (**Figure 2D**). This process led to the identification of two genes, POSTN and VCAN, associated with OS pathogenesis, and the VCAN gene was selected as the focal point for the subsequent phases of analysis.

GSEA

GSEA showed that VCAN was mainly enriched in ECM Proteoglycans (**Figure 3A**), NABA Matrisome (**Figure 3B**), NABA Core Matrisome (**Figure 3C**), and ECM Organization signaling pathways (**Figure 3D**).

Gene expression data analysis

The expression profile of VCAN was investigated across multiple cancer types within TCGA using TIMER2 for analysis. VCAN was found to be overexpressed in breast invasive carcinoma (BRCA), cholangiocellular carcinoma (CHOL), colorectal adenocarcinoma (COAD), esophageal carcinoma (ESCA), head and neck squamous cell carcinoma (HNSC), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD),

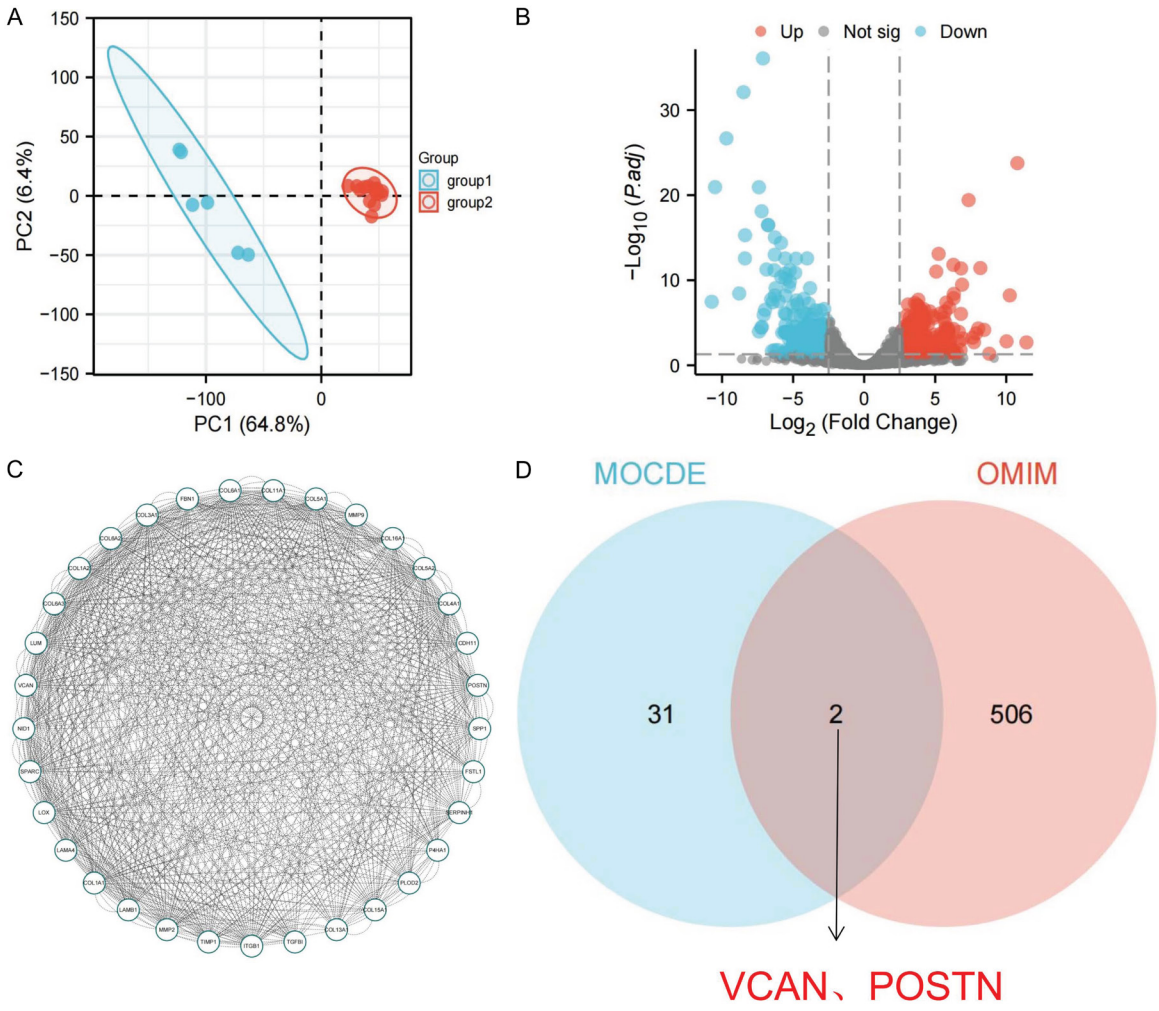

Figure 2. OS Core Gene Screening. A. Data homogenization process; B. Blue indicates down-regulated genes, and red indicates up-regulated genes; C. OS core modules; D. Core module intersects with OMIM dataset to get POSTN and VCAN genes. OS, osteosarcoma; VCAN, Versican.

lung squamous cell carcinoma (LUSC), rectum adenocarcinoma (READ), skin cutaneous melanoma (SKCM), stomach adenocarcinoma (STAD), and thyroid carcinoma (THCA) compared to that in normal tissues (P<0.05). Conversely, its expression was lower in kidney chromophobe (KICH) compared to that of normal tissues (P<0.05). Since some normal data information was missing from the TCGA database, normal tissues from the GTEx database were introduced as a reference, and VCAN was found to be lowly expressed in KICH, ACC, DLBC, PCPG, and THYM tumors, and showed high expression in all other tumors (P<0.05). We also analyzed the protein level of VCAN, which found low expression in glioblastoma and endometrioid carcinoma, and high expression in all other tumors (P<0.05) (**Figure 4**).

The HEPIA2 "pathological staging map" module was employed to observe the correlation between VCAN expression and the pathological stages of cancers, including ACC, BLCA, CESC, COAD, KICH, LUAD, PAAD, and THCA, and showed a progressive increasing trend (**Figure 5**).

Survival analysis

We divided cancer cases into high and low expression groups according to the level of VCAN. TCGA and GEO datasets were used to investigate the correlation between VCAN expression and the prognosis of patients with different tumors. In the TCGA database, high expression of VCAN was associated with ACC (P=0.042), LIHC (P=0.037), MESO (P=0.0014),

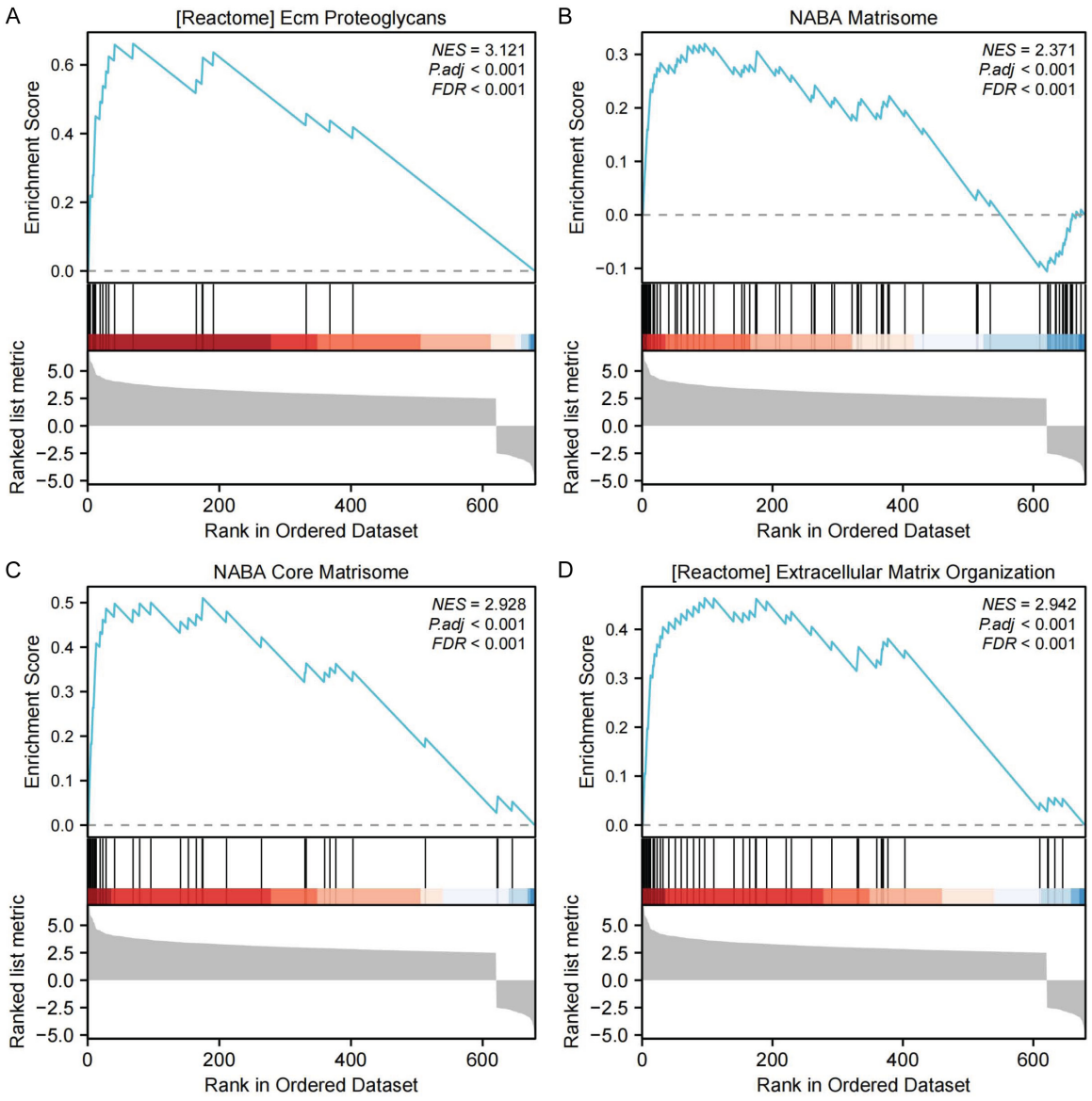

Figure 3. GSEA enrichment analysis. A. ECM Proteoglycans signaling pathways; B. NABA Matrisome signaling pathways; C. NABA Core Matrisome signaling pathways; D. Extracellular Matrix Organization signaling pathways. ECM, extracellular matrix.

STAD (P=0.0016), UVM (P=0.015), and BLCA (P=0.03) (**Figure 6**). Data from disease-free survival analysis revealed correlations between high SND1 expression and unfavorable prognosis in CESC (P=0.046), STAD (P=0.014), and UVM (P=0.016). However, there was no observable correlation between low SND1 expression and disease-free survival in testicular germ cell tumors (TGCT) (P>0.05) (**Figure 7**).

Genetic variation analysis data

We observed the genetic alteration patterns of VCAN in different tumor samples within the TCGA cohort. As shown in **Figure 8A**, the highest frequency of SND1 mutations (19.14%) was found in patients with cutaneous melanoma with "mutations" as the primary type. "Amplified" copy number alteration was the predominant type of adrenocortical carcinoma, with an alteration frequency of approximately 3.3% (**Figure 8A**). Notably, cases with genetic alterations (2.23%) of ovarian plasmacytic cystic adenocarcinoma had copy number deletions of VCAN (**Figure 8A**). The main genetic alteration type in VCAN involved missense mutations, particularly mutations like X3355_splice, P3355S, and P3355T in the Lectin-C

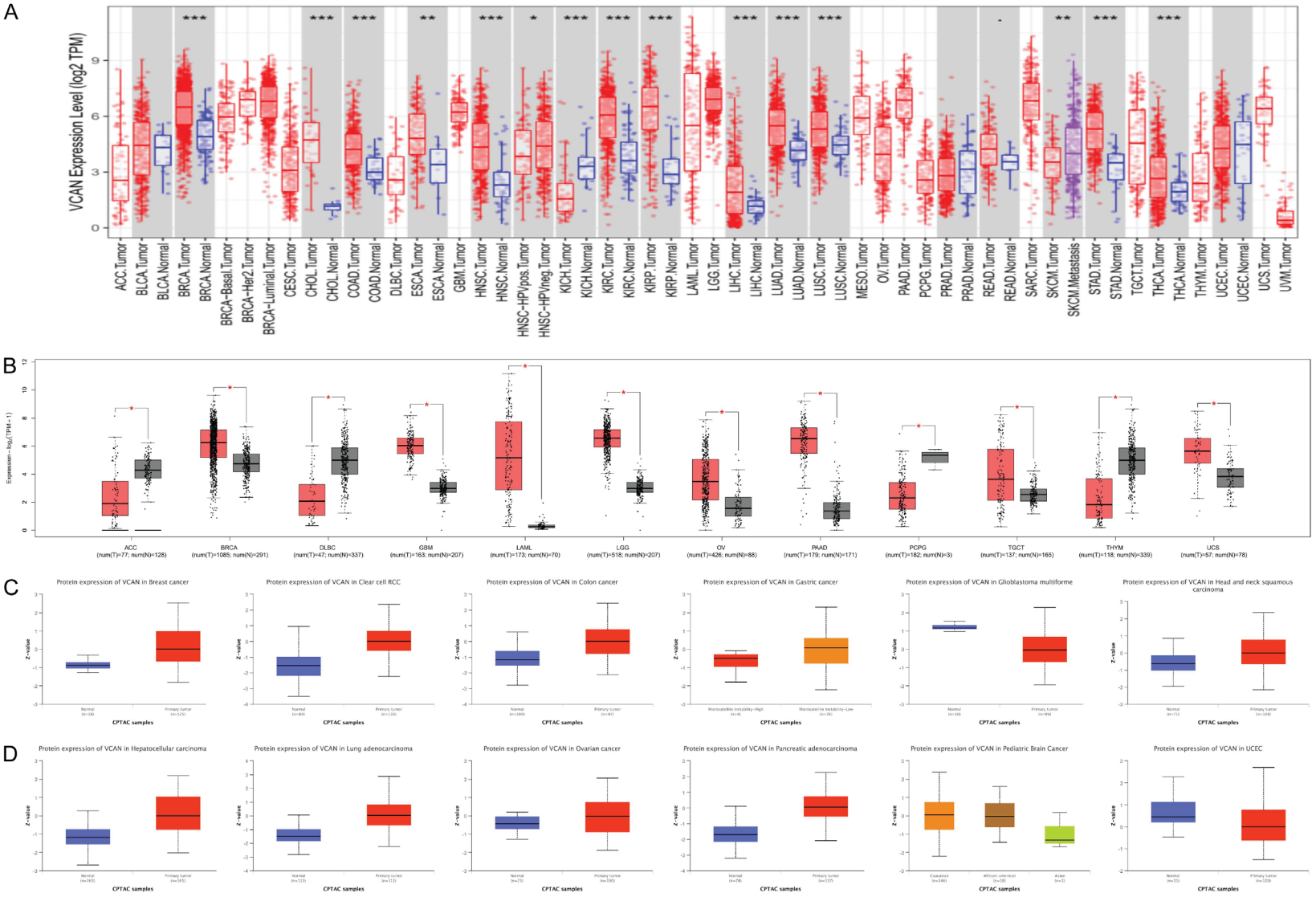

Figure 4. Gene expression data analysis. A. VCAN gene expression in different cancers or specific cancer subtypes by TIMER2 analysis, *P<0.05; **P<0.01; ***P<0.001; B. For adrenocortical carcinoma (ACC), ovarian cancer (OV), BRCA, DLBC, GBM, LAML, LGG, OV, PAAD, PCPG, TGCT, THYMHE, and UCS type tumors in the TCGA project, corresponding normal tissues in the GTEx database were used as controls, *P<0.05; C. Based on the CPTAC dataset, the expression of total VCAN protein was analyzed in breast cancer, renal clear cell carcinoma, colon cancer, gastric cancer, glioblastoma, head and neck squamous cell carcinoma, hepatocellular carcinoma, lung cancer, ovarian cancer, pancreatic cancer, pediatric brain cancer gastric verrucous carcinoma, and endometrioid carcinoma. D. Based on TCGA data, ACC, BLCA, CESC, COAD, KICH, LUAD, PAAD, and THCA were analyzed with expression levels of Log2(TPM+1). DLBC, diffuse large B-cell lymphoma; TGCT, testicular germ cell tumors; COAD, colorectal adenocarcinoma; KICH, kidney chromophobe; LUAD, lung adenocarcinoma; THCA, thyroid carcinoma.

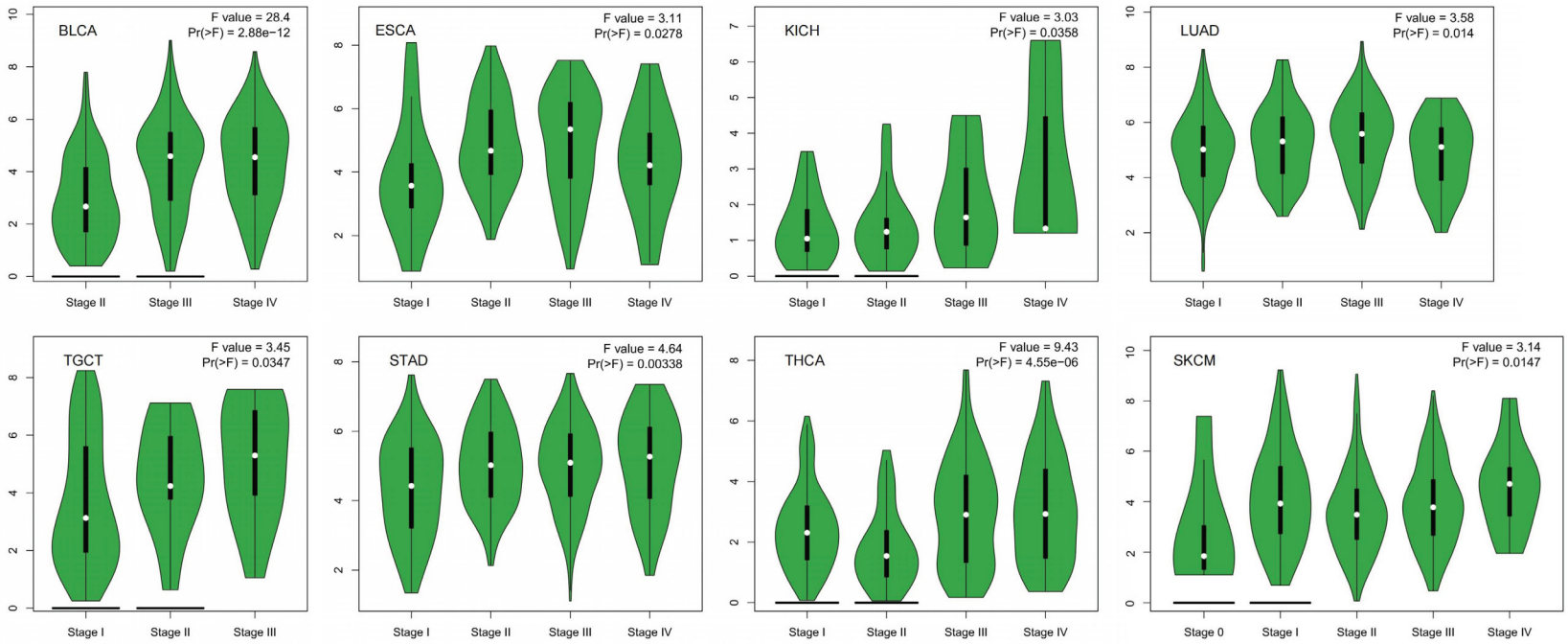

Figure 5. Expression levels of VCAN genes in different tumors and pathological stages. VCAN, Versican.

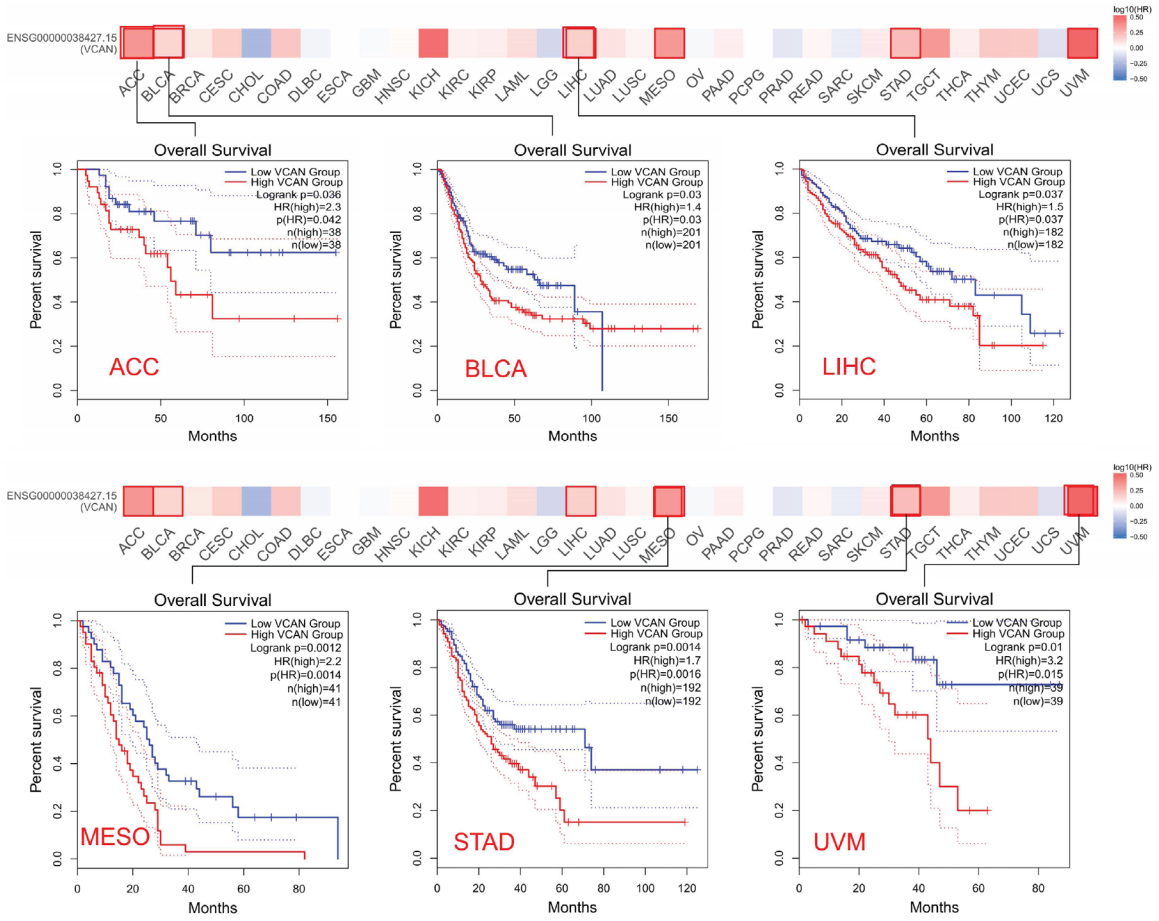

Figure 6. Correlation between VCAN gene expression and overall survival. VCAN, Versican.

domain of the SND1 gene. These mutations were detected in two cases of endometrial cancer, one case of gastric adenocarcinoma, one case of rectal adenocarcinoma, and one case of cervical squamous cell carcinoma (**Figure 8B**). The X3355 site in the 3D structure of the VCAN protein was observed (**Figure 8C**). In addition, we explored the potential association between VCAN gene alterations and clinical survival prognosis in different types of cancer cases. The data in **Figure 8D** reveal that UCEC cases with altered VCAN exhibited better prognosis (P=0.0309), disease-specific survival (P=0.0425), and progression-free survival (P=3.11e-3), but a contrary trend in disease-free survival (P=0.125), compared to cases without altered VCAN (**Figure 8D**).

Immuno-infiltration analysis

Tumor-infiltrating immune cells, as an important component of the tumor microenvironment (TME), are closely associated with the development, progression, or metastasis of cancers. Cancer-associated fibroblasts in the TME stroma have been reported to be involved in regulating the function of various tumor-infiltrating immune cells [24, 25]. Here, we used the EP-IC, MCPCOUNTER, XCLL, and TIDE algorithms to investigate the potential relationship between the level of infiltration of different immune cells and the expression of VCAN in different cancers. We observed a strong positive correlation between VCAN expression and a range of cancer types including ACC, BLCA, BRCA, BRCA-Basal, BRCA-Her2, BRCA-LumA, BRCA-LumB, CESC, COAD, ESCA, HNSC, HNSC-HPC-, HNSC-HPC+, KIRC, LIHC, LUAD, LUSC, OV, PAAD, PCPG, PRADA, READ, SARC, SKCM, SKCM-Metastasis, STAD, TGCT, THCA, THYM, and UCEC (P<0.05). No negative correlation was observed with the related tumors (**Figures 9** and **10**).

VCAN gene enrichment analysis

To further investigate the molecular mechanisms of VCAN genes in tumorigenesis, we

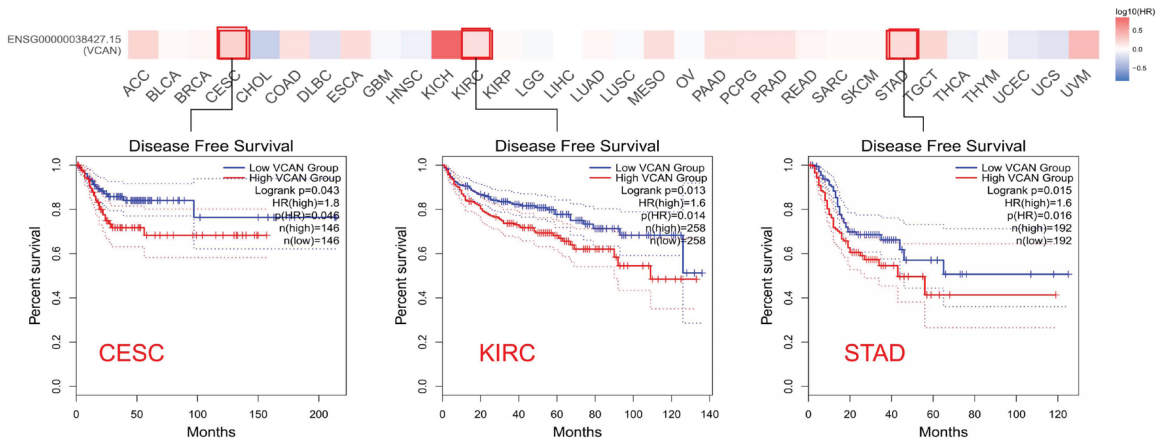

Figure 7. Correlation between VCAN gene expression and disease-free survival. VCAN, Versican.

screened relevant genes targeting VCAN-binding proteins and VCAN expression for pathway enrichment analysis. Based on the STRING tool, we obtained a total of 42 VCAN-binding proteins and developed protein interaction networks (**Figure 11A**). The GEPIA2 tool was used to combine all tumor expression data from TCGA to obtain the top 100 genes associated with VCAN expression. A co-expressed gene, FBN1, was obtained by taking the intersection of the above two groups of genes (**Figure 11B**). As shown in **Figure 11C**, VCAN expression levels were positively correlated with Anthrax Toxin Receptor 1 (ANTXR1, R=0.53), type V collagen A2 (COL5A2, R=0.5), chondroitin sulfate N-acetylgalactosaminyltransferase 2 (CSGALNACT2, R=0.49), and cysteine acidic secretory protein (SPARC, R=0.48) (**Figure 11C**). Corresponding heat map data also showed that VCAN was positively associated with the above five genes in most detailed cancer types (**Figure 11D**). Cross-tabulation analysis of the above two groups revealed a common member, FBN1. GO and KEGG enrichment analysis of the binding and expression-related proteins of VCAN revealed that BP function was mainly enriched in ECM tissue (ECM organization). CC function was mainly enriched in the collagen-containing ECM. MF function was mainly enriched in ECM structural constituent. The KEGG pathway was mainly enriched in signaling pathways, such as ECM-receptor interaction. See **Figure 11E**.

Discussion

In this study, we adopted an integrated approach involving mRNA microarrays to validate that non-tumor tissues exhibit differential expression compared to OS tumor tissue. The OMIM database was used to intersect with the core module, and VCAN was found to be a core gene for OS. GSEA revealed that VCAN was mainly enriched in the ECM signaling pathway during OS pathogenesis, which is consistent with the pathogenesis of OS. Previous studies [12-16] have suggested that VCAN is closely associated with the occurrence and metastasis of many malignant tumors, such as gastric cancer, liver cancer, kidney cancer, rectal cancer, and lung cancer. However, no existing study has explored the interplay between VCAN and OS development. Furthermore, there is a dearth of comprehensive pan-cancer analyses focusing on VCAN from a holistic perspective. Therefore, in this study, we screened the core genes of OS and performed a comprehensive analysis of VCAN in different tumors from the perspectives of gene expression and gene variants based on HPA, TCGA, Kap-Lan-MeierPlotter and other databases to reveal the association between OS and various cancers.

In vertebrate organisms, tissue structure and cellular behaviors are regulated by a microenvironment containing cellular and molecular elements, of which ECM is a representative microenvironment. ECM constitutes a complex network of supramolecular aggregates that are extracellularly secreted. It is composed of various components including extracellular proteins, proteoglycans, enzymes, and glycoproteins. It has a wide range of uses, mainly involving structural scaffolding and biochemical support of cells and tissues, and ECM homeostasis is essential for normal organ development and

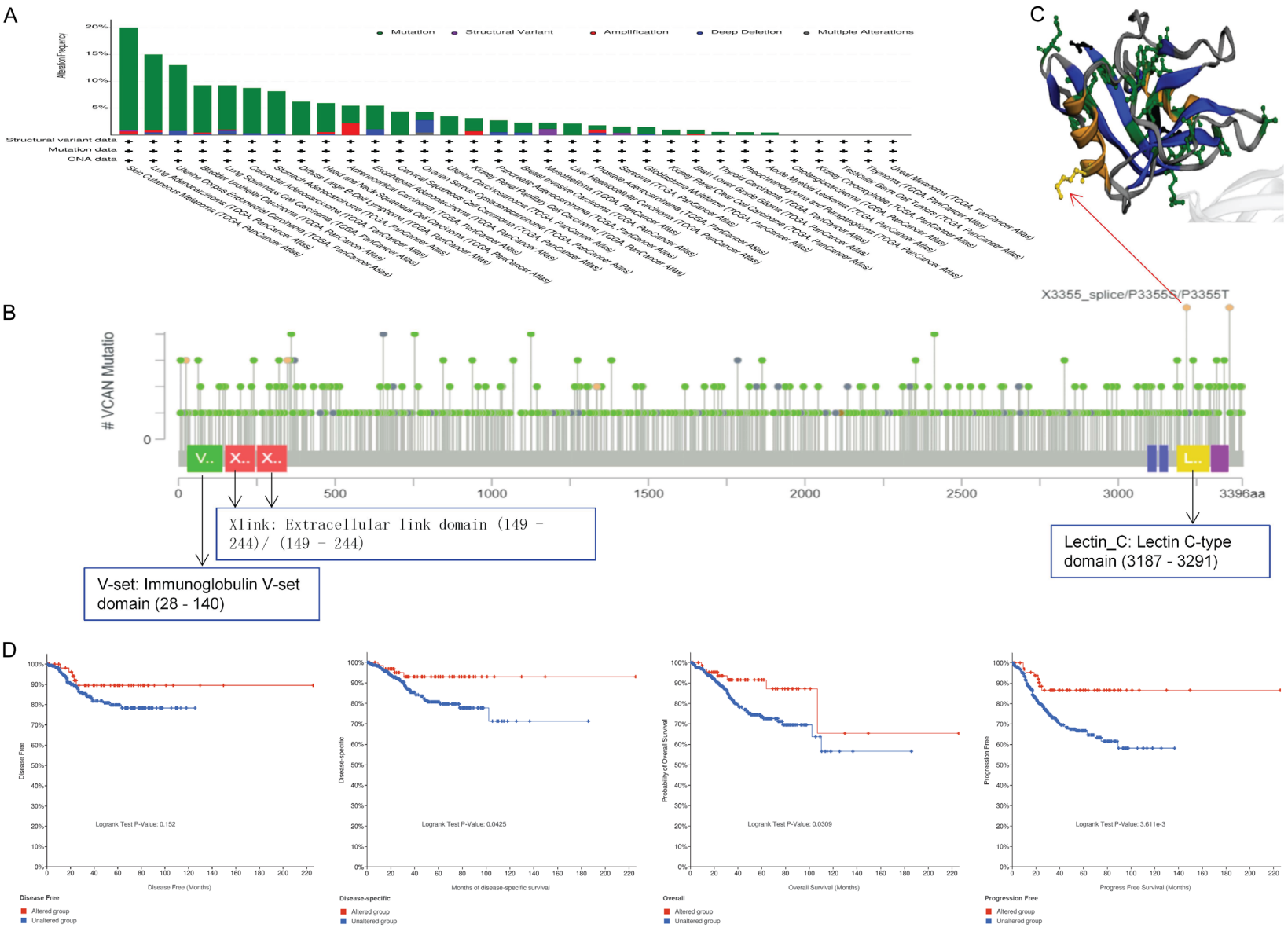

Figure 8. Genetic variation analysis. A. Mutation types of VCAN in different tumor samples; B. Mutation sites; C. 3D map of mutation sites with high mutation frequency (X3355_splice/P3355S/P3355T); D. Potential correlation between mutational status and overall, disease-specific, disease-free and progression-free survival in UCEC. VCAN, Versican.

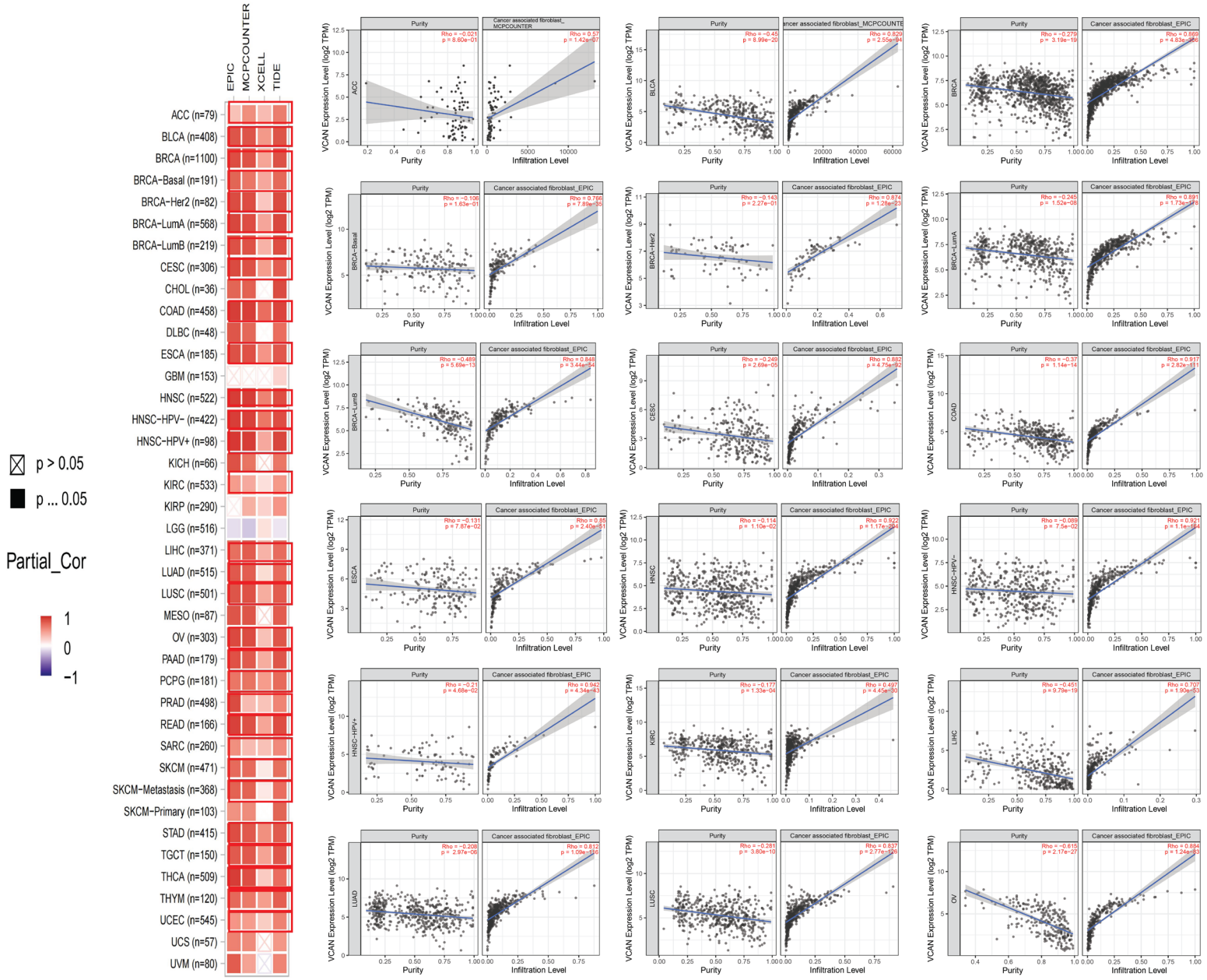

Figure 9. Correlation analysis between VCAN expression levels and immune infiltration of CAFs. VCAN, Versican.

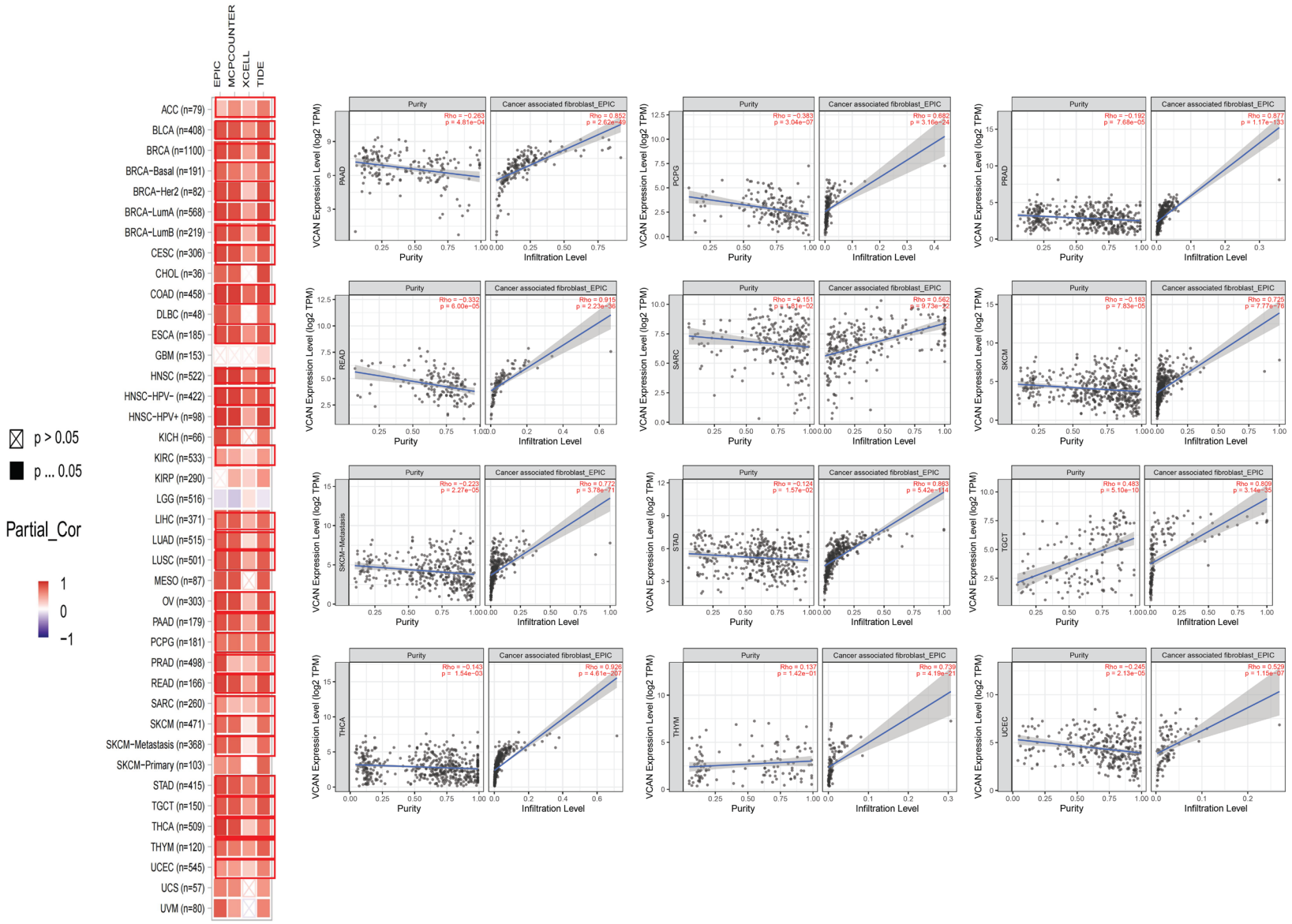

Figure 10. Correlation analysis between VCAN expression levels and immune infiltration of CAFs. VCAN, Versican.

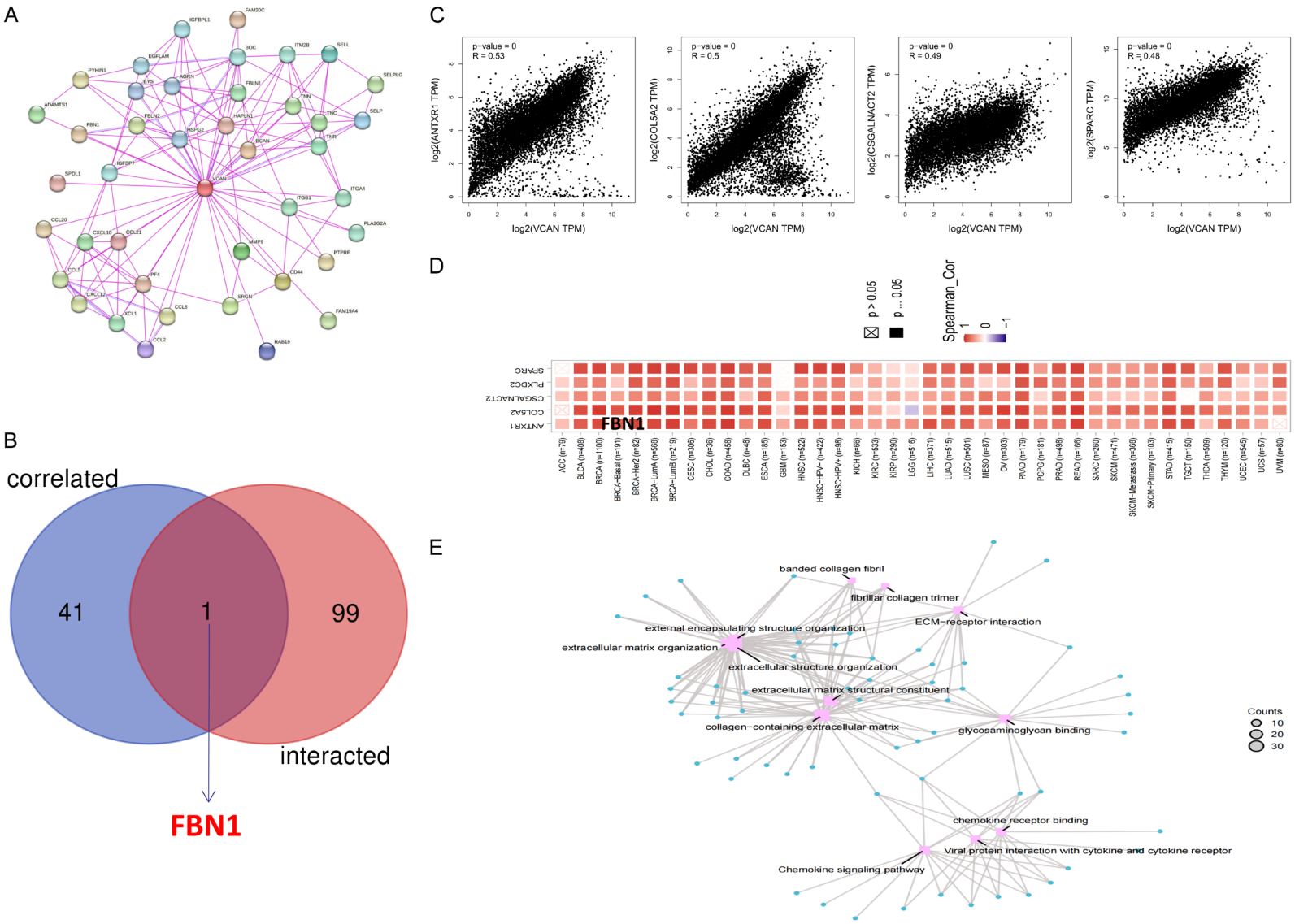

Figure 11. VCAN-related gene enrichment analysis. A. VCAN binding proteins were obtained using the STRING tool; B. Cross-tabulations of VCAN binding and related genes were performed to obtain the FBN1 gene; C. Using the GEPIA2 method, the top 100 VCAN-related genes from the TCGA project were obtained and the expression correlations between VCAN and selected target genes, including ANTXR1, COL5A2, CSGALNACT2, and SPARC, were analyzed; D. The corresponding heat map data in the cancer type; E. GO and KEGG pathway analysis based on VCAN binding and interacting genes. VCAN, Versican.

function. In addition, its persistent alteration or dysregulation can lead to pathological changes such as tumorigenesis [17, 18]. ECM is tightly controlled during embryonic development and organ homeostasis, yet in cancer, ECM is often dysregulated and disorganized, and abnormal ECM can promote cell invasion and metastasis to influence cancer progression and continuously remodel itself [19]. The ECM of tumors coming from distant metastases remodels in a manner that promotes cancer cell implantation. Remodeling is regulated at multiple levels by VCAN, and enzymes involved in ECM remodeling have become a hallmark of malignant metastasis and recurrence. These enzymes play a pivotal role in these processes, offering diagnostic and histological applications [20, 21]. Previous studies have found that tumors such as breast, lung, prostate, colon, bladder, kidney, melanoma, and ovarian cancers commonly develop bone metastases and have been shown to have ECM components involved in these cancerous metastatic processes. Among these ECM components, VCAN has been identified as a major key player in tumor metastasis and recurrence [22-24]. VCAN can interact with a variety of ECM ligands and cell surface molecules to promote multiple functions in tumor cells [25, 26]. In cancerous tumors, VCAN binds to a variety of proteins and carbohydrates in the ECM with different structural domains, and its main ligand is hyaluronic acid, whose production and upregulation in the TME are associated with tumor metastasis and recurrence [27]. Components of the ECM, such as acetyl hyaluronate and VCAN, are often highly expressed around cancerous cells, allowing cancer cells to evade surveillance by natural killer cells and other immune cells as well as resist antibody-dependent cytotoxic effects [28, 29]. In addition to this, VCAN produced by cancer cells can alter the phenotype of innate and acquired immune cells, causing the associated immune cells in ECM to secrete pro-oncogenic inflammatory factors, such as TNF-α to promote tumor progression, and IL-6 to reduce the killing power of dendritic cells against tumor cells [30, 31]. Moreover, VCAN-associated conjugates can exhibit variations based on the specific isoforms of VCAN present and be stabilized through interactions with different linker proteins (e.g., HAPLN1), which influence tumor invasiveness, and the variability among VCAN conjugates complicates the mechanisms of tumor invasion and recurrence [32, 33]. In summary, the VCAN-ECM-related action pathway plays an important role in the recurrence and metastasis of a variety of cancerous tumors.

OS progression is also influenced by changes in TME or ECM filled with various cytokines, with transforming growth factor-β (TGF-β) being one of the most abundant cytokines in TME [34]. VCAN, an ECM-associated gene, encodes a large chondroitin sulfate proteoglycan that accumulates in the tumor mesenchyme, interacts with other ECM components to influence tumor progression, and plays a key role in tumor metastasis and recurrence [35-37]. Previous studies have identified four possible splice isoforms of VCAN (V0-V3), with V0 and V1 being the most commonly expressed isoforms in OS, both of which contribute to OS metastasis and recurrence [38]. V0 and V1 are often overexpressed in advanced OS and are considered markers of OS metastasis [39]. TGF-β was found to regulate the expression of VCAN in normal and tumor cells. In OS and recurrent breast cancer, cells can engage in autocrine signaling involving TGF-β1. This autocrine loop prompts cancer-associated fibroblasts to overexpress VCAN, and regulate the overexpression of VCAN isoforms V0 and V1 within ECM through miR-143. This intricate interplay contributes to the induction of OS invasion and metastasis [40-42]. Related studies have found that bone ECM consists of an organic compartment and an inorganic compartment. The organic compartment includes type I collagen, proteoglycans, and glycoproteins. Bone tissue itself is a complex static lattice structure embedded and surrounded by a dense network of bone ECM, which is involved in processes, such as differentiation and metastasis in the life cycle of bone cells [43, 44]. Bone ECM is particularly conducive to bone metastasis. Many malignant tumors tend to metastasize to bone tissue, and metastatic cancer cells interact with osteoblasts to disrupt bone ECM homeostasis and induce bone ECM remodeling, which in turn causes osteolytic bone metastasis [45]. When osteolytic changes occur, bone tissue extravasates. Also, cancer cells become less invasive and adopt the expression of surface markers characteristic of osteoblasts or osteoclasts, allowing cancer cells to achieve immune escape and making OS treatment less effective [46]. Moreover, cancer cells in metastatic tumors

secrete enzymes that modify the composition and structure of the bone ECM. Enzymes like lysyl oxidase and histone K are involved in this process, leading to ECM remodeling. This remodeling is characterized by the emergence of invasive pseudopods, increased tissue stiffness, augmented connective tissue proliferation, and destruction of the basement membrane [47-49]. With bone ECM remodeling, stimulation of osteolytic bone metastasis is accompanied by cytokines such as TGF-β embedded in bone ECM, which stimulates cancer cell proliferation and induces ECM remodeling [50]. This "vicious cycle" promotes osteolysis and induces bone ECM remodeling, causing changes in VCAN and ECM, as well as impairing the effectiveness of treatment for metastatic bone tumors [51, 52].

In summary, the effects of ECM remodeling and aberrant VCAN expression on tumor cell migration and invasion are evident. With changes in the TME, OS often undergoes osteolytic changes - the VCAN-ECM "vicious circle" makes the survival rate of patients with metastatic and recurrent OS significantly lower. In addition, the VCAN-ECM pathway plays an important role in the development, metastasis, and recurrence of pan-cancer. However, it is still unclear whether there is a genetic-level association between OS and pancreatic cancer. Therefore, it is necessary to further investigate the key genes and their molecular networks that jointly influence the development of OS and pan-cancer. Elucidating the role of VCAN-ECM-related pathways holds the potential to bridge the knowledge gap between OS and pan-cancer dynamics. This could provide new ideas for the early diagnosis of OS and the formulation of innovative therapeutic targets and strategies for OS.

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Disclosure of conflict of interest

None.

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