Comprehensive analysis and immunohistochemistry localization of NRP1 expression in pancancer and normal individual tissues in relation to SARS-CoV-2 susceptibility

JIEWEN FU[1,2*], JIAYUE HE[1*], LIANMEI ZHANG[1,3*], JINGLIANG CHENG[1], PENGFEI ZHANG[4], CHUNLI WEI[1], JUNJIANG FU[1] and DABING LI[1,2]

[1]Key Laboratory of Epigenetics and Oncology, The Research Center for Preclinical Medicine, Southwest Medical University; [2]School of Basic Medical Sciences, Southwest Medical University, Luzhou, Sichuan 646000; [3]Department of Pathology, The Affiliated Huai'an No. 1 People's Hospital of Nanjing Medical University, Huai'an, Jiangsu 223300; [4]National Health Commission Key Laboratory of Cancer Proteomics, Department of Oncology, Xiangya Hospital, Central South University, Changsha, Hunan 410008, P.R. China

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Abstract. Neuropilin 1 (NRP1/CD304) is a typical membrane-bound co-receptor for vascular endothelial growth factor, semaphorin family members and viral severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). However, NRP1 expression levels across cancer types and the potential role of SARS-CoV-2 infection in patients with cancer are not clear. Online databases, such as The Cancer Genome Atlas database of Human Protein Atlas, Gene Expression Profiling Interactive Analysis and cBioPortal were used for the expression analysis in this study. Immunohistochemical (IHC) staining for NRP1 was performed in the tissues of patients with non-small cell carcinoma. As a result, it was found that NRP1 mRNA and protein expression levels were highest in the female reproductive tissues and the respiratory system, specifically in the nasopharynx, bronchus and fallopian tube, as well as in adipocytes, hepatic stellate cells, Sertoli cells, endothelial cells and dendritic cells. IHC showed that the NRP1 protein was mainly localized to the cytoplasm and membrane in the tissues of patients with non-small cell carcinoma, demonstrating its role in lung infection by SARS-CoV-2, due to invasion of cell membranes by the virus. Levels of NRP1 mRNA were significantly increased in lymphoid neoplasm diffuse large B-cell lymphoma, esophageal carcinoma, glioblastoma multiforme, head and neck squamous cell carcinoma, kidney renal clear cell carcinoma (KIRC), pancreatic adenocarcinoma, stomach adenocarcinoma and thymoma, and significantly decreased in cervical squamous cell carcinoma and endocervical adenocarcinoma, kidney chromophobe, lung squamous cell carcinoma, ovarian serous cystadenocarcinoma, uterine corpus endometrial carcinoma and uterine carcinosarcoma, compared with corresponding healthy tissues in pancancer, indicating roles for viral invasion in most cancer types. Moreover, low NRP1 expression was significantly associated with long overall survival (OS) time in adrenocortical carcinoma, brain lower grade glioma, stomach adenocarcinoma and uveal melanoma, but with short OS time in KIRC only. The ENST00000374867.6 (NRP1-202) isoform is most highly expressed in most cancer types and thus could be involved in tumorigenesis and SARS-CoV-2 invasion in cancer patients. NRP1 may be involved in SARS-CoV-2 invasion in patients with cancer, including those with lung cancer.

Introduction

Neuropilin 1 (NRP1; OMIM: 602069), also known as CD304/VEGF165R/NRP/vascular endothelial cell growth factor 165 receptor, is a typical membrane-bound co-receptor both for members of the semaphorin family and vascular endothelial growth factor (VEGF) (1-4). The *NRP1* gene is located at human chromosome 10p11.22. NRP1 encodes a deduced 923-amino acid protein with a molecular mass of 103,134 Da (NM_003873.7, NP_003864.5) containing an N-terminal signal sequence, a transmembrane region, an ectodomain and a cytoplasmic domain, consistent with the structure of cell surface receptors (5). These specific domains participate in different signaling pathways and have versatile roles in controlling survival, migration, invasion, angiogenesis and axon guidance through ligands binding to co-receptors, including VEGF and semaphorin family members (3,4).

Correspondence to: Professor Junjiang Fu or Professor Dabing Li, Key Laboratory of Epigenetics and Oncology, The Research Center for Preclinical Medicine, Southwest Medical University, 3-319 Zhongshan Road, Luzhou, Sichuan 646000, P.R. China
E-mail: fujunjiang@swmu.edu.cn
E-mail: lidabing@swmu.edu.cn

*Contributed equally

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Two landmark papers by Cantuti-Castelvetri *et al* (6) and Daly *et al* (7) found that NRP1 can act as a receptor to facilitate severe acute respiratory syndrome coronavirus 2 (SARS CoV-2) invasion into host cells (8). Mutation of novel NRP1 interaction sites located in the vestigial plasminogen-apple-nematode (PAN) domain was recently reported to reduce SARS-CoV-2 S-protein internalization (9). SARS-CoV-2 causes severe coronavirus disease 2019 (COVID-19), which has been the leading global pandemic since outbreaks began at the end of 2019. Unlike the S-protein of SARS-CoV-1, the S-protein of SARS-CoV-2 has a polybasic sequence domain (Arg-Arg-Ala-Arg) (the C-end rule) at the S1-S2 boundary that facilitates cleavage by furin (10), an enzyme convertase that catalyzes conversion of a substance to its active state. Thus, SARS-CoV-2 can easily enter host cells with the aid of NRP1, promoting its infectivity and tropism (11). In addition, cells from bronchoalveolar lavage fluid of patients with COVID-19, but not uninfected cells, show an increase in NRP1 RNA expression in SARS-CoV-2-positive cells (6), further enhancing SARS-CoV-2 entry. Wang *et al* (12) reported that NRP1 is highly expressed in macrophages and dendritic cells (DCs) of myeloid lineage but not in $CD4^+$ T cells, acting as an inhibitor of human immunodeficiency virus-1 infectivity. Targeting NRP1 is a potential approach to preventing SARS-CoV-2 entry (13,14) and developing potential antitumor drugs (15,16), with peptide-based inhibition of angiogenesis, proliferation and migration in tumor cells (17). In addition, NRP1 facilitates the invasion and replication of other various viruses, such as herpesvirus Epstein-Barr virus (EBV) (18), pseudorabies virus (19), mouse cytomegalovirus (20), and the human T-cell lymphotropic virus-1 (HTLV-1) and HTLV-2 retroviruses (21).

Small-molecule inhibitors of the S-protein of SARS-CoV-2 may bind to NRP1 (22). *In silico* analysis has revealed that natural product small molecules that interfere with SARS-CoV-2 binding to NRP1 are potential candidate novel antiviral agents (23-26). Folic acid, leucovorin and alimemazine may have the potential to prevent SARS-CoV-2 internalization by interacting with the S-protein/NRP1 complex (27,28). Targeting NRP1 with small molecules thus has the potential to interfere with SARS-CoV-2 invasion (29). However, the potential of NRP1 expression in SARS-CoV-2-infected patients with all types of cancer is not clear. It is essential to identify novel small molecules from natural products or traditional Chinese medicine with antitumor functions that can modulate expression of host cell entry regulators to interfere with SARS-CoV-2 entry (11,22,30). The present study analyzed NRP1 expression, DNA mutations and prognosis with different levels of NRP1 expression across cancer types, and susceptibility to SARS-CoV-2 invasion.

Materials and methods

Online databases. Human *NRP1* gene expression in normal tissues and cancer was analyzed in The Cancer Genome Atlas (TCGA) database of the Human Protein Atlas (HPA) (https://www.proteinatlas.org/ENSG00000099250-NRP1/tissueand(https://www.proteinatlas.org/ENSG00000099250-NRP1/pathology) (31,32), and the association between *NRP*1 gene expression and survival in patients with cancer was analyzed by Gene Expression Profiling Interactive Analysis (GEPIA 2; http://gepia2.cancer-pku.cn/#analysis) (33,34) in TCGA and GTEx data. Mutation and survival analyses for NRP1 across multiple cancer types were conducted using cBioPortal (https://www.cbioportal.org/results/cancerTypesSummary?case_set_id=all&gene_list=NRP1&cancer_study_list=5c8a7d55e4b046111fee2296) (35-38). For these analyses, the term 'NRP1' was used in the online systems.

Antibodies and reagents. NRP1 antibody was purchased from Santa Cruz Biotechnology, Inc. (cat. no. sc-5307). 3,3'-Diaminobenzidine (DAB Substrate System; cat. no. ZLI-9017) was purchased from Origene Technologies, Inc.

Immunohistochemistry (IHC) for NRP1. The non-small cell carcinoma tissues were collected from a resection specimen from a 78-year-old male patient treated in Affiliated Huai'an No. 1 People's Hospital of Nanjing Medical University Huai'an City, with informed consent. The tissues from patients with lung cancer were fixed in 10% neutral formalin for 24 h at room temperature. Paraffin sections (5 μm) were deparaffinized in xylene and rehydrated using a descending alcohol series (100, 95, 85 and 70%). The sections were immersed in 10 μM sodium citrate buffer, and heated at 98˚C for 12 min for antigen retrieval. Following washing in 1X PBS, slides were incubated with 3% hydrogen peroxide for 10 min, washed in PBS again, and covered with blocking serum (5% bovine serum albumin) for 30 min at room temperature prior to incubation overnight at 4˚C with the primary antibody diluted at 1:200. The sections were sequentially washed in PBS, incubated with the biotin-conjugated secondary antibody (cat. no. SP-9000; ready-to-use; Origene Technologies, Inc.) for 60 min, incubated with the streptavidin-conjugated horseradish peroxidase (HRP) for 10 min and finally with the DAB Substrate System. The sections were then counterstained with hematoxylin for 20 sec at room temperature, dehydrated using an ascending alcohol series (70, 85, 95 and 100%), cleared with xylene and mounted with neutral balsam. Images were captured under a light microscope (30).

Statistical analysis. Statistical analysis was performed using an unpaired t-test (two groups) with SPSS v25.0 software, and data are expressed as the mean ± standard deviation. $P<0.01$ was considered to indicate a statistically significant difference. Kaplan-Meier curves and the log-rank test were also used (http://gepia2.cancer-pku.cn/#survival).

Results

NRP1 expression in human normal tissues, including immune cells. The expression levels for viral receptors might play important roles in SARS-CoV-2 susceptibility in normal individual tissues. The present study analyzed NRP1 expression levels using the HPA and the results are shown in Fig. 1. *NRP1* mRNA and protein were mainly expressed in tissues of the female reproductive tract, followed by the respiratory system, muscle tissues, bone marrow and lymphoid tissues, endocrine tissues, liver and gallbladder, kidney and urinary bladder, male reproductive tissues, gastrointestinal tract and pancreas,

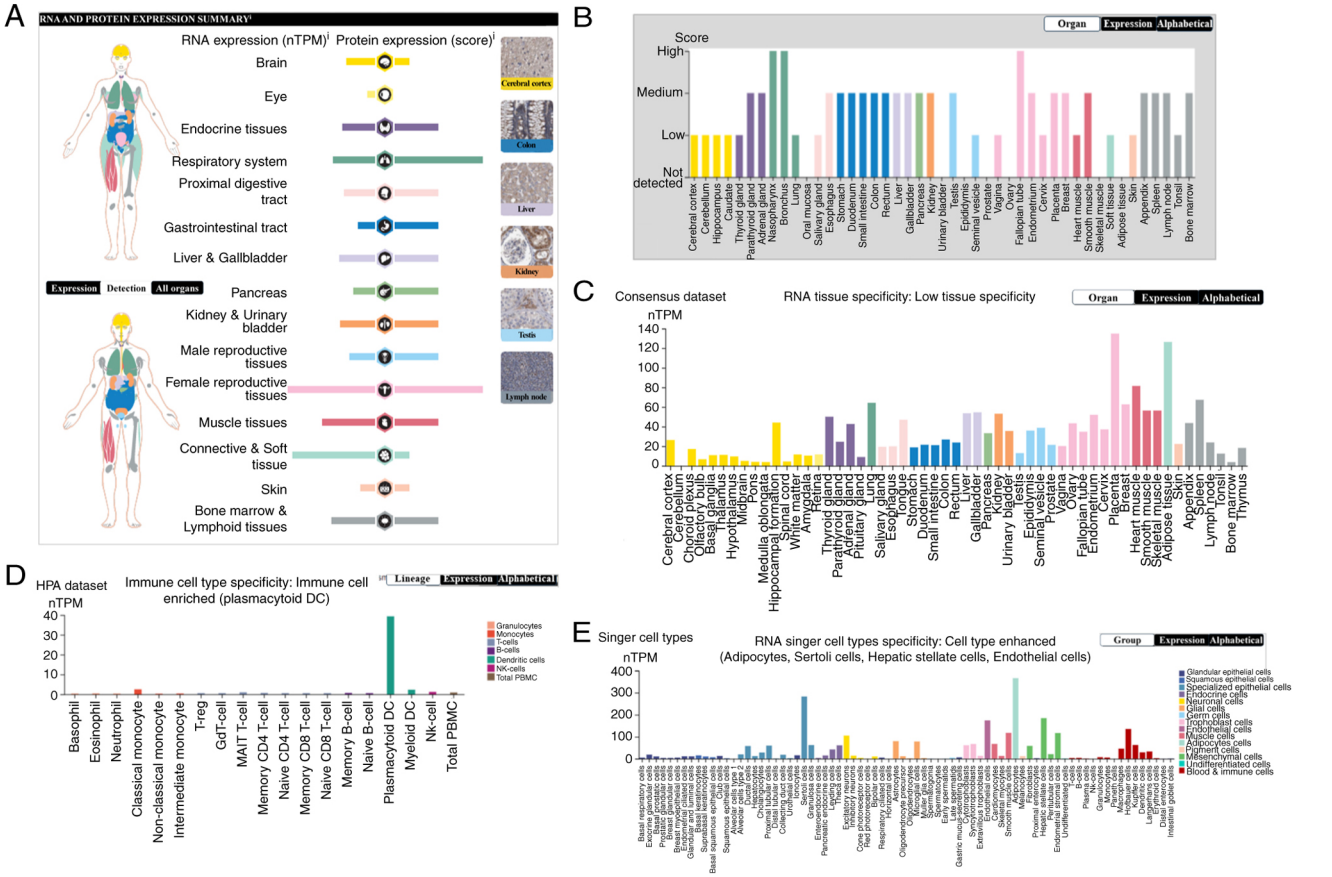

Figure 1. NRP1 expression in tissues of healthy human individuals. (A) Overview of NRP1 mRNA and protein expression. (B) NRP1 protein expression in healthy tissues. (C) NRP1 mRNA expression in healthy tissues. (D) NRP1 mRNA expression for immune cell types in the HPA dataset. (E) NRP1 mRNA expression in single cell types. NRP1, neuropilin 1; nTPM, normalized transcripts per million; HPA, Human Protein Atlas; DC, dendritic cell; NK, natural killer; PBMC, peripheral blood mononuclear cells.

with low or no expression in other tissues. For connective and soft tissue, and the brain, the *NRP1* mRNA levels were high whereas the protein levels were relatively low (Fig. 1A). Specifically, the NRP1 protein expression was highest in the nasopharynx and bronchus (respiratory system), and fallopian tube (female reproductive tissue) (Fig. 1B), whereas the *NRP1* mRNA expression was highest in the placenta [133.7 normalized transcript per million (nTPM), female reproductive tissue] and adipose tissue (125.2 nTPM, connective and soft tissue) (Fig. 1C). The *NRP1* mRNA expression in immune cells was analyzed and found to be enriched in plasmacytoid DCs (39.3 nTPM, dendritic cells); other cells had no or very low levels of *NRP1* mRNA expression (Fig. 1D). Finally, the *NRP* mRNA levels in single cell types were enhanced, including adipocytes (366.0 nTPM), Sertoli cells (282.7 nTPM), hepatic stellate cells (184.6 nTPM) and endothelial cells (174.4 nTPM) (Fig. 1E). Overall, NRP1 is most highly expressed in the female reproductive tissues and the respiratory system, specifically in the nasopharynx, bronchus and fallopian tube, as well as in adipocytes, hepatic stellate cells, Sertoli cells, endothelial cells and dendritic cells.

NRP1 expression in cancer tissues and corresponding healthy tissues of different cancer types. Patients with malignant cancer are more vulnerable to SARS-CoV-2 attack, leading to high mortality rates (39-42). Expression levels of NRP1 between tumor tissues and corresponding healthy tissues among different cancer types were analyzed using GEPIA 2, and the results are shown in Fig. 2. The levels of *NRP1* were significantly increased in lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), head and neck squamous cell carcinoma (HNSC), kidney renal clear cell carcinoma (KIRC), pancreatic adenocarcinoma (PAAD), stomach adenocarcinoma (STAD) and thymoma (THYM), and significantly decreased in cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), kidney chromophobe (KICH), lung squamous cell carcinoma (LUSC), ovarian serous cystadenocarcinoma (OV), uterine corpus endometrial carcinoma (UCEC) and uterine carcinosarcoma (UCS) compared with matched healthy tissues in different cancer types (TCGA normal and GTEx data) (Fig. 2A-C). These findings indicate roles for viral invasion in most cancer types, especially in DLBC, ESCA, GBM, HNSC, KIRC, PAAD, STAD and THYM.

IHC results for non-small cell carcinoma stained by NRP1. IHC was performed to assess NRP1 expression and localization in the tissues of patients with non-small cell carcinoma, and representative results are shown in Fig. 3. The NRP1 protein was mainly localized to the cytoplasm and membrane (Fig. 3A and B). Panel C shows the control without NRP1

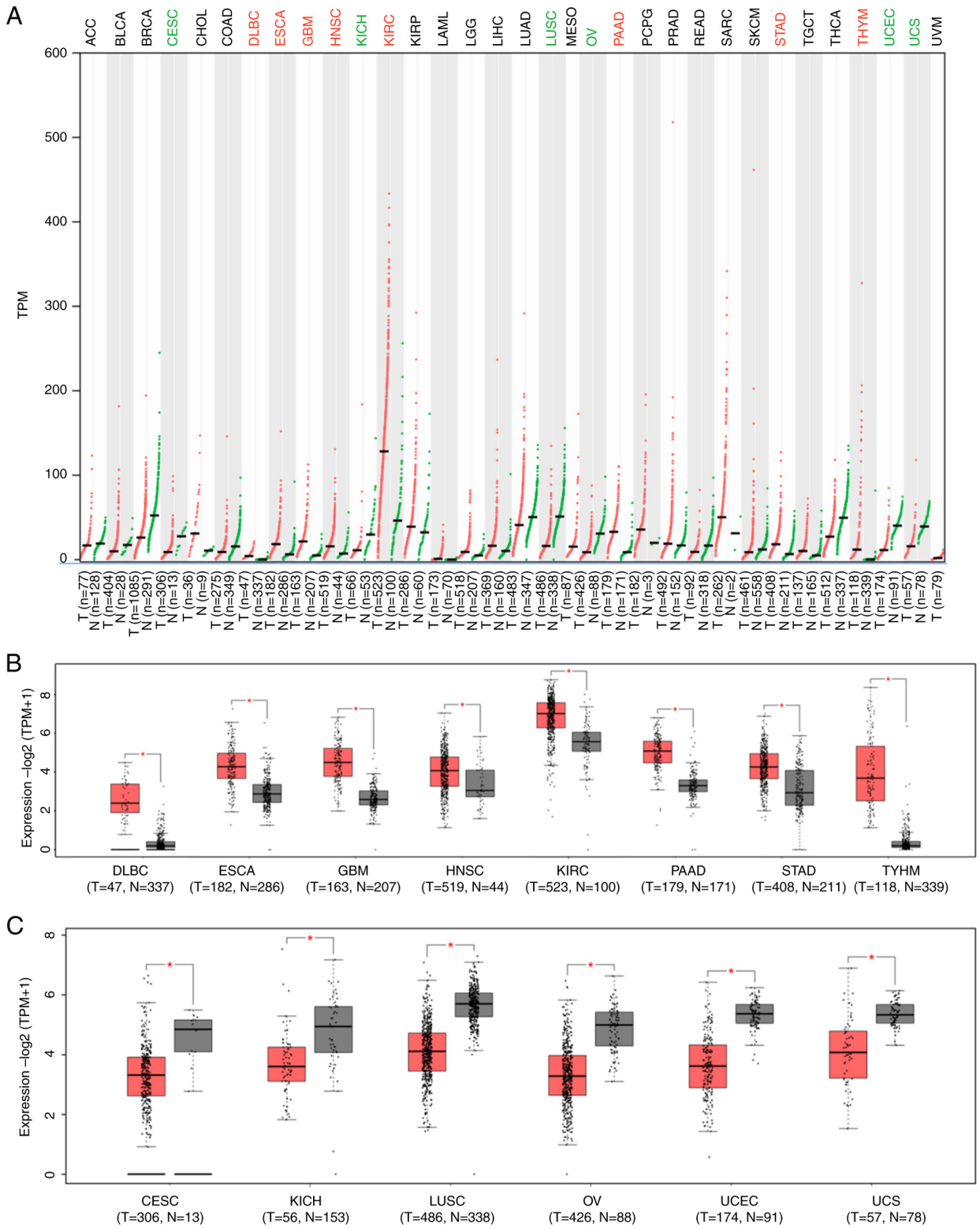

Figure 2. NRP1 expression comparison between cancer tissues and matched healthy tissues. (A) NRP1 expression comparison between human tumor tissues and matched healthy tissues among different cancer types. Red colors indicate increase of expression while green colors indicate decrease of expression in tumor tissues in The Cancer Genome Atlas database. (B) NRP1 expression increases significantly in cancer tissues compared with that in matched healthy tissues among different cancer types. (C) NRP1 expression is significantly decreased in cancer tissues compared with that in matched healthy tissues among different cancer types. *P<0.01. N, normal; T, tumor; NRP1, neuropilin 1; TPM, transcripts per million. ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.

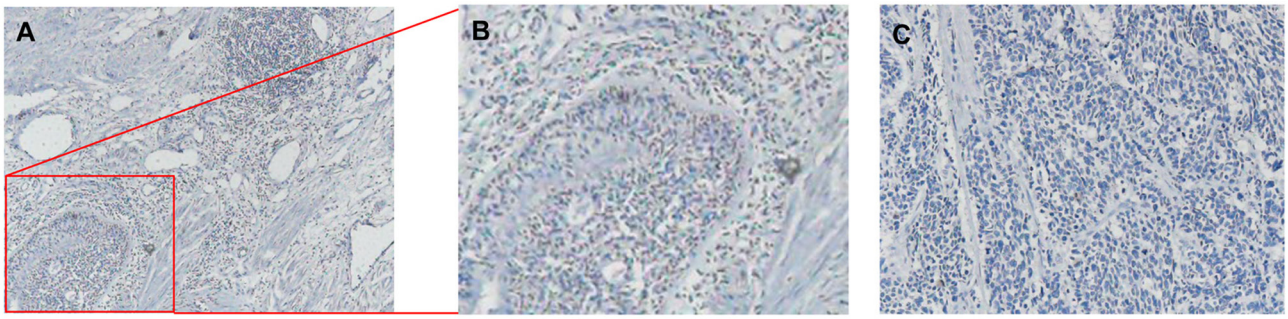

Figure 3. IHC results for non-small cell carcinoma tissues stained for NRP1. IHC results for non-small cell carcinoma stained with NRP1 antibody at (A) x40 magnification and (B) at x200 magnification, as an enlarged version of the highlighted area in (A). (C) Control without NRP1 antibody staining at x200 magnification. NRP1, neuropilin 1; IHC, immunohistochemistry.

antibody staining of the tissues from the patients with non-small cell carcinoma (Fig. 3C).

Prognostic value of the NRP1 expression in different cancer types. The prognostic value of *NRP1* was further explored with the median used as the cutoff value between the high and low expression groups. Low expression was significantly associated with long OS time in adrenocortical carcinoma (ACC), brain lower grade glioma (LGG), stomach adenocarcinoma (STAD) and uveal melanoma (UVM) (Fig. 4A, C, E and G), as a P-value of <0.01 is being used for significance, implying that NRP1 may be an unfavorable marker. CESC, GBM and LUSC showed a trend but P-values were between 0.01 and 0.05 (Fig. 4B, F and G). However, low expression of NRP1 was significantly associated with a short OS time in KIRC only (Fig. 4I), implying that NRP1 may be favorable. The survival map of NRP1 expression among different cancer types is summarized in Fig. 4J.

Distribution and structure of NRP1 isoforms among cancer types. Different isoforms have different domains and roles that may be responsible for SARS-CoV-2 entry (43,44). *NRP1* has 14 isoforms in different cancer types with differential expression levels (Fig. 5A). Expression levels of isoforms ENST00000374875.5 (NRP1-002) and ENST00000374867.6 (NRP1-202) were highest in most of the cancer types, followed by ENST00000395995.5 (NRP1-203), ENST00000413802.1 (NRP1-009) and ENST00000418675.5 (NRP1-008); others were very low or not detectable (Fig. 5A). Consistently, the isoform utilization for ENST00000374867.6 (NRP1-202) was highest, followed by ENST00000374875.5 (NRP1-002), across all 33 cancer types; others were very low (Fig. 5B). The genomic structures of NRP1 isoforms from 33 different cancer types are shown in Fig. 5C. The isoforms NRP1-001, NRP1-202 and NRP1-203 have CUB, DUF3481, F5_F8_type_C and MAM domains. NRP1-001 and NRP1-202 are 923 amino acids long, and NRP1-203 is 906 amino acids long, but the others lack some or all functional domains (Fig. 5C). Homologs of the *NRP1* gene are conserved in chimpanzee, Rhesus monkey, mouse, rat, dog, cow, chicken, zebrafish and frog, with functional domain and size in humans being the same as NRP1-001 and NRP1-202 in cancer (Fig. 5D). These results indicate that the isoform ENST00000374867.6 (NRP1-202) might be involved in normal development in humans, in tumorigenesis and in SARS-CoV-2 entry in patients with different cancer types.

Mutations in NRP1 across cancer types. Mutation of *NRP1* interaction sites, located in the PAN domain, was recently reported to reduce SARS-CoV-2 S-protein internalization (9). Mutations at any of the first three cysteines (C82A, C104A and C147A) of the *NRP1* gene had significant negative impacts on SARS-CoV-2 S-protein binding. Thus, the present study aimed to determine which *NRP1* mutations occur in pan-cancer tissues. In 32 cancer types from 10,953 patients, a total of 201 *NRP1* mutations, including missense, truncating, splice and structural variation/fusion mutations, were found; skin cutaneous melanoma showed the highest mutation frequency in 9.91% of 444 cases, followed by UCEC in 7.18% of 529 cases (Fig. 6A). The detailed *NRP1* mutation landscapes appeared to be distributed across whole-gene regions, with missense mutations being dominant (Fig. 6B). However, there were no *NRP1* mutations at the following three cysteines: C82, C104 and C147.

Next, the survival predictive value was analyzed, and the association with survival between groups with altered and unaltered NRP1 showed no significant difference, including with regard to overall survival (OS). The median OS time of the unaltered group was 79.46 months (95% CI, 73.68-84.20), while that of the altered group was shorter, at 65.33 months (95% CI, 48.76-107.18) (Fig. 6C).

Discussion

Cantuti-Castelvetri *et al* (6) and Daly *et al* (7) first found that NRP1 could act as a receptor to facilitate SARS-CoV-2 invasion into host cells (8). Recently, Lu *et al* (45) identified NRP1 as an entry receptor for Kaposi's sarcoma-associated herpesvirus (KSHV), a double-stranded DNA virus, in mesenchymal stem cells. KSHV has been implicated in the pathogenesis of KS (46) and other malignancies, including multicentric Castleman's disease (47), primary effusion lymphoma (48) and childhood osteosarcoma (49), highlighting NRP1 as a risk factor for viral entry in patients with cancer and viral-associated endemic cancer. However, NRP1 expression across cancer types and the potential roles of SARS-CoV-2 infection in patients with cancer are not clear.

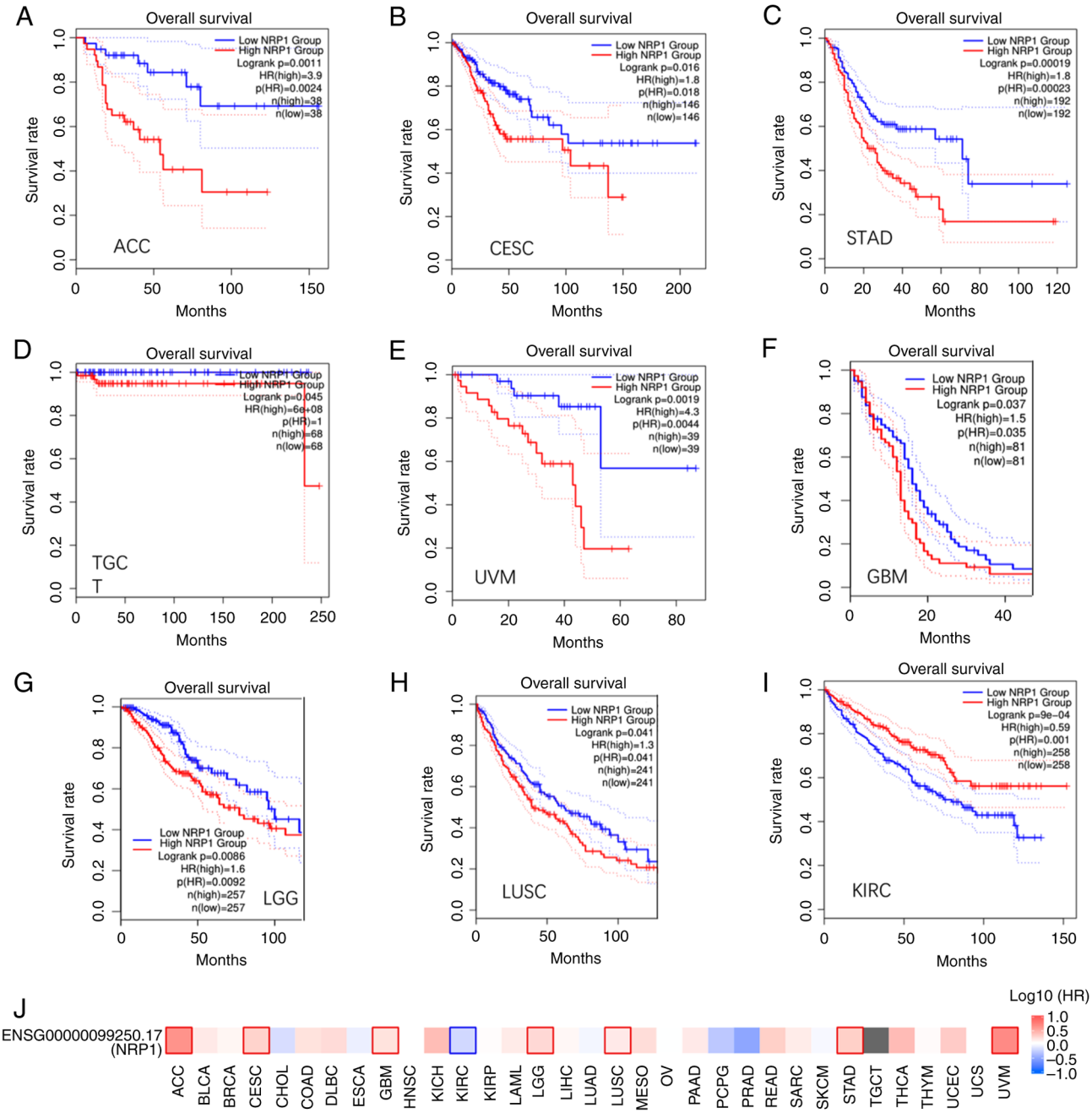

Figure 4. Overall survival analysis across multiple cancer types. (A-I) OS results based on NRP1 expression and plotted Kaplan-Meier curves for (A) ACC, (B) CESC, (C) STAD, (D) TGCT, (E) UVM, (F) GBM, (G) LGG, (H) LUSC and (I) KIRC. (J) Survival map for NRP1 expression in different cancer types. NRP1, neuropilin 1. ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.

It is therefore important to investigate NRP1 expression across cancer types and the potential roles of SARS-CoV-2 in patients infected with cancer. In the current study, *NRP1* mRNA and protein expression was found to be highest in the female reproductive tissues and the respiratory system, specifically in the nasopharynx, bronchus and fallopian tube, as well as in adipocytes, hepatic stellate cells, Sertoli cells, endothelial cells and dendritic cells in the immune system. IHC showed that the NRP1 protein was mainly localized to the cytoplasm and membrane in the tissues of patients with non-small cell carcinoma, demonstrating its role in lung infection by SARS-CoV-2. The levels of *NRP1* were significantly increased in DLBC, ESCA, GBM, HNSC, KIRC, PAAD, STAD and THYM and significantly decreased in

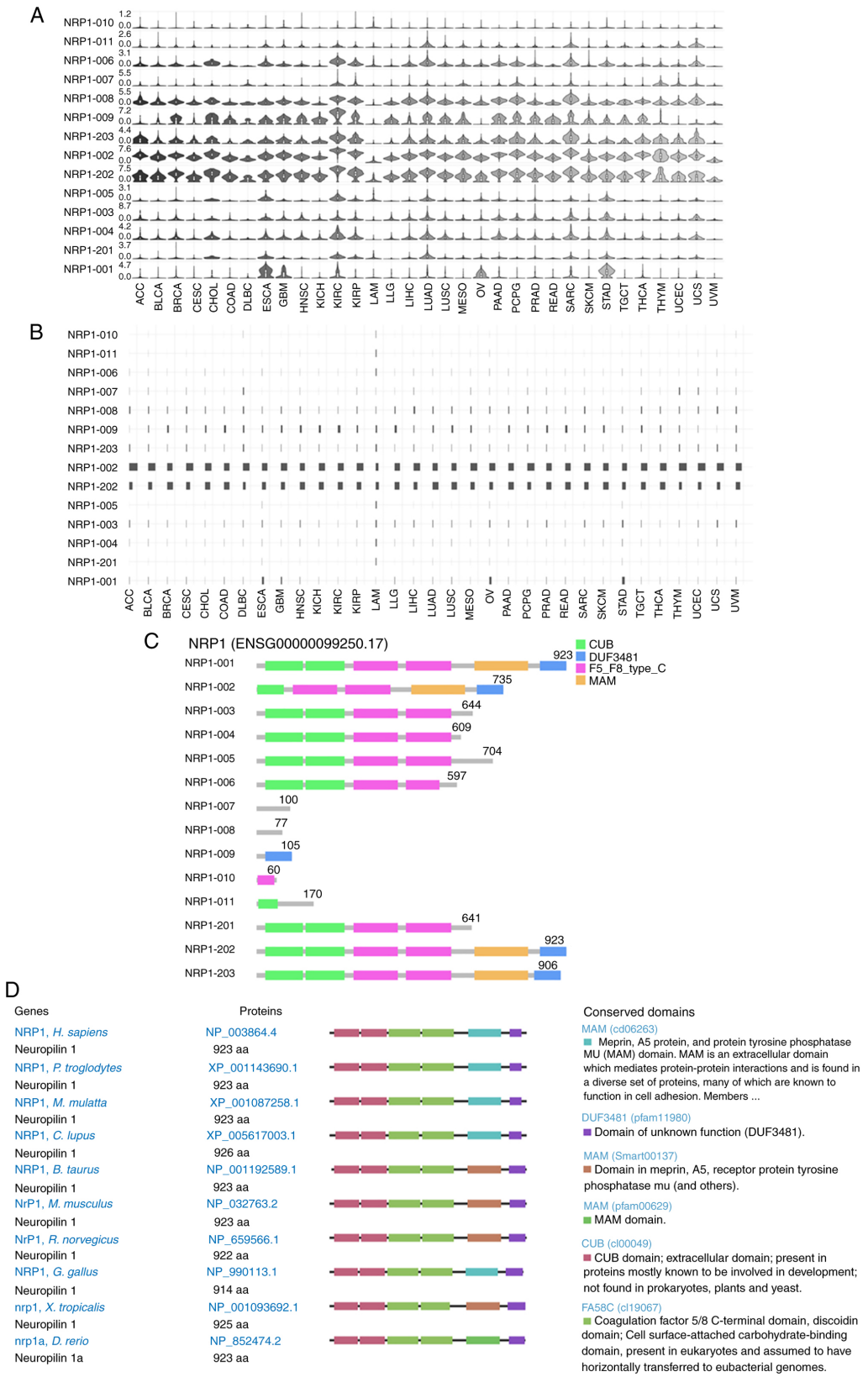

Figure 5. NRP1 expression distribution, isoform usage and conservation. (A) Profiles for NRP1 expression distribution in violin plots and (B) isoform usage in bar plots among different cancer types. (C) NRP1 structure in multiple cancer types. (D) NRP1 conservation in different species. NRP1, neuropilin 1. ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.

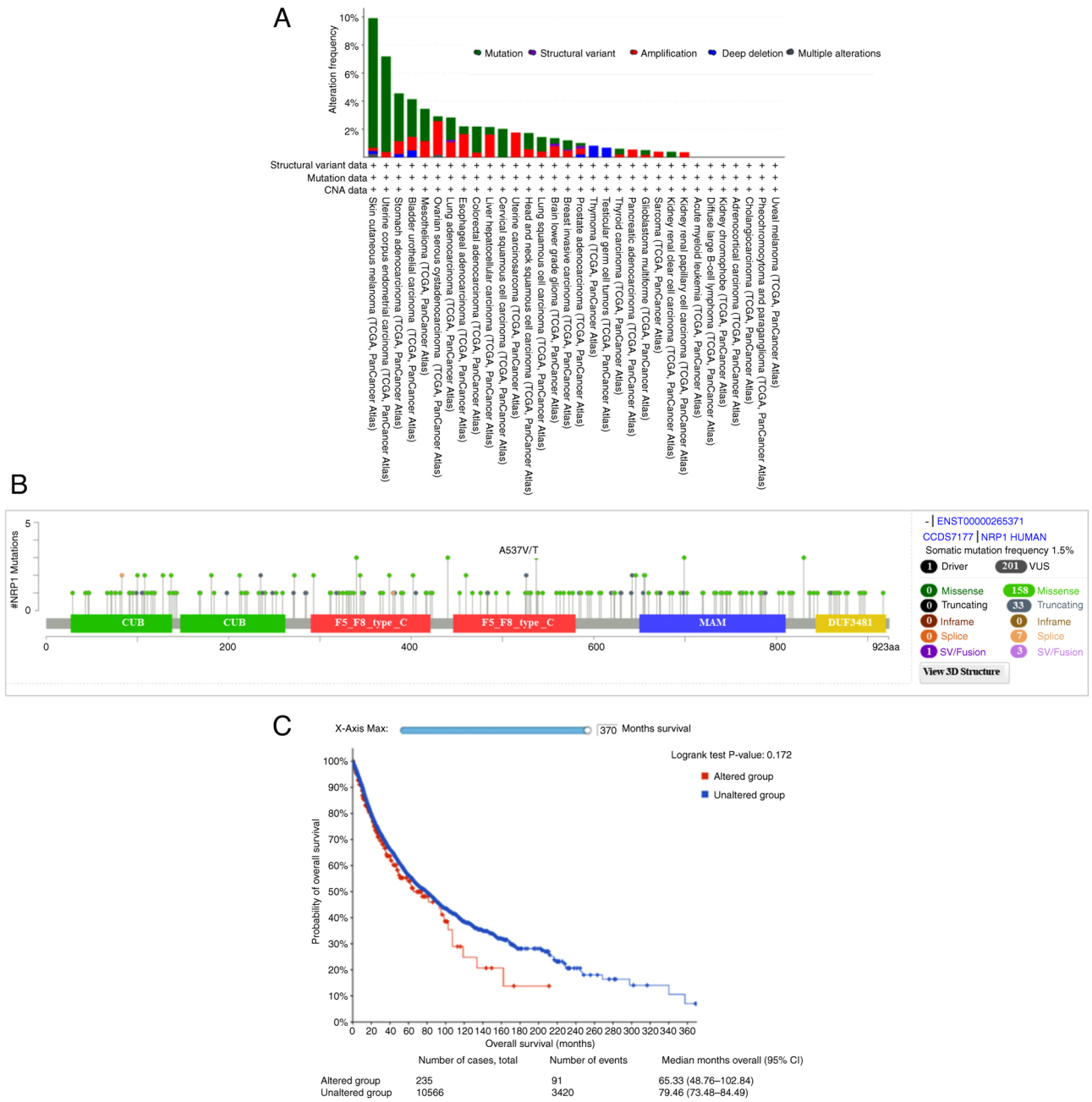

Figure 6. Mutations in the *NRP1* gene across multiple cancer types. (A) Mutation frequency of *NRP1* across multiple cancer types. (B) Mutation locations of *NRP1* across multiple cancer types. (C) Overall survival for wild-type and mutant *NRP1* cases. NRP1, neuropilin 1; TCGA, The Cancer Genome Atlas; CNA, copy number alteration; SV, structured variant; VUS, variant of uncertain significance.

CESC, KICH, LUSC, OV, UCEC and UCS, when compared with matched healthy tissues in different cancer types, indicating roles for viral invasion in most cancer types. Overall, low expression of ACC, LGG, STAD and UVM was significantly associated with longer OS time, but with shorter OS time in KIRC only, demonstrating that a poor prognosis was associated with high NRP1 expression in most cancer types. Notably, Morin *et al* (50) reported that NRP1 low expression is a biomarker of improved survival in patients with KIRC/renal cell carcinoma (RCC), thus confirming the bioinformatics results. In addition, NRP1 expression in KIRC/RCC patients might enhance infectivity or disease severity and the oncolytic properties of SARS-CoV-2 (51). The isoform ENST00000374867.6 (NRP1-202) is highly expressed in most cancer types and thus might be involved in tumorigenesis and SARS-CoV-2 invasion in patients with different cancer types.

The limitations of the present study are based on the bioinformatics approach, and experimental validation of NRP1 expression may need to be performed in addition to IHC. Future studies will explore whether natural products, such as cordycepin and thymoquinone, would inhibit NRP1 expression and prevent susceptibility to SARS-CoV-2, as well as other viruses, such as EBV, KSHV, HTLV-1 and HTLV-2.

In conclusion, the present study highlights the significance of NRP1 expression, DNA mutation and prognostics in different cancer types and matched healthy tissue, and susceptibility to SARS-CoV-2 entry, and promotes the clinical potential and

practical implications of therapy for viral diseases, including COVID-19, and cancer by targeting NRP1.

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Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JiF, JH, LZ, CW and JC performed the experimental studies, data acquisition, data analysis and literature search. JuF collected and analyzed the data. JuF, DL and PZ designed and supervised the project. DL and JC confirm the authenticity of all the raw data. JuF wrote and edited the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethical Committee of Southwest Medical University (Luzhou, China) (approval no. 20221117-049). Written informed patient consent was obtained for participation.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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