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Epitope mapping of severe acute respiratory syndrome-related coronavirus nucleocapsid protein with a rabbit monoclonal antibody

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ABSTRACT

The emergency SARS-CoV-2, a member of severe acute respiratory syndrome-related coronaviruses (SARSr-CoV), is still greatly harming the health of mankind. SARS-CoV-2-specific monoclonal antibodies (MAbs), which can identify SARS-CoV-2 from common human coronaviruses, are considered to extensively apply to developing rapid and reliable antigen assays. In this study we generated a rabbit MAb (RAb) detecting SARS-CoV-2 nucleocapsid protein (NP), which has cross-reaction with SARS-CoV-1 NP, but not with NPs of MERS and common human CoVs (OC43, NL63, 229E, and HKU1). With truncated NP fragments and synthesized peptides, the linear epitope detected by RAb was mapped in peptide N4-8, 393–407 amino acid residue (TLLPAADLDDFSKQL) of SARS-CoV-2 NP. This epitope N4-8 was highly conserved in SARSr-CoVs, including SARS-CoV-2, SARS-CoV-1, and bat CoV RaTG13 strain. However, the corresponding peptide of bat SARSr-CoV BtKY72 strain could not be recognized by RAb, which indicates amino acid D399 may be critical for N4-8 epitope detected by RAb. The present study will be conducive to developing reliable diagnosis for SARS-CoV-2 and gaining insights into the function of the SARS-CoV-2 N protein.

1. Introduction

The 2019 novel coronavirus (2019-nCoV, SARS-CoV-2) has caused the global outbreaks of 2019 coronavirus disease (COVID-19) (Huang et al., 2020). SARS-CoV-2, assigned to the species severe acute respiratory syndrome-related coronavirus (SARSr-CoV) (Gorbalenya et al., 2020), represents the third zoonotic CoV introduced to humans after SARS-CoV-1 and MERS-CoV earlier in the twenty-first century. SARS-CoV-2 refers to the seventh CoV, which is transmissible between human beings (with other four of HCoV-229E, HCoV−OC43, HCoV-NL63 and HCoV-HKU1) (Salata et al., 2019; Zhou et al., 2020). Globally, as of 31 January 2021, 102,083,344 confirmed cases of COVID-19, including 2,209,195 deaths, have been reported to WHO (WHO, 2020).

Rapid, low-cost and accurate point-of-care diagnostic tests (POCTs) aiming to detect viral antigens in clinical samples are urgently required for mass-screening of potential SARS-CoV-2 infected cases, timely isolation of infected cases, and effectively controlling the disease (Rosenthal, 2020; Vandenberg et al., 2020; Binnicker, 2020; Ji et al., 2020; Porte et al., 2020). In addition, SARS-CoV-2-specific monoclonal antibodies (MAbs) are recognized to extensively apply to developing such antigen assays. The nucleocapsid protein (NP) packages the virus genome RNA in the coronavirus envelope (D'Cruz et al., 2020). The coronavirus NP refers to the predominant viral structural protein detectable in clinical samples of nasopharyngeal aspirate, gargle solution, serum, faecal material and urine from patients (Chan et al., 2020; Xiang et al., 2020). The NP exhibits strong immunogenicity, and it is highly conserved, which is a promising target of antigen detection for early screening of SARS-CoV-2-infected cases (Kim et al., 2020; Panda et al., 2020; Tung and Limtung, 2020). Subsequently, NP-specific MAbs will be useful for proposing rapid and reliable SARS-CoV-2 NP testing methods. Moreover, the identification of epitopes is of high importance for developing rapid diagnostic reagents.

A rabbit monoclonal antibody (RAb) was employed to detect SARS-CoV-2 NP and SARS-CoV-1 NP, and an investigation was conducted on the epitope recognized by this RAb and the cross-reactivity among

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human coronaviruses. The present study will be conducive to the development of reliable and stable diagnosis for SARS-CoV-2 infection.

2. Materials and methods

2.1. Vectors, recombinant proteins and peptides

The cDNAs of SARS-CoV-2 NP open reading frame (ORF) were synthetized by Shanghai Generay Biotech Co., Ltd (Shanghai, China), which were directly cloned into pcDNA3.1(+) vector digested with restriction enzymes BamHI and NotI, termed as pcDNA-SARS-CoV-2-NP.

Prokaryotic expression system was employed to express NP proteins. In addition, the hexahistidine (His) tags expression vector pQE30 was adopted to express different coronavirus full length NP proteins (e.g., SARS-CoV-2-NP, SARS-CoV-1-NP and OC43-NP), and the glutathione of S-transferase (GST) expression vector pGEX-4T-3 was applied for the production of truncated NP fragments with an N-terminal GST tag. The GST fusion proteins were purified with GST-Bind Resin (Novagen, USA) by complying with the manufacturer's instructions. Moreover, the products were analysed by performing SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The primers for cloning were shown in Table 1.

The short peptides of 12–15 amino acids were synthesized by GL Biochem (Shanghai) Co. Ltd (Shanghai, China). The peptides were purified by high-performance liquid chromatography to 85 % purity, of which the identity was determined by conducting the mass spectrometry analysis. Next, the peptides were dissolved in water to 1 mg/mL and stored in aliquots to avoid repetitive freeze-thaw cycles.

Recombinant MERS-NP, NL63-NP, 229E-NP and HKU1-NP proteins expressed in *E.coli* were offered by Asbio Technology, Inc. (Guangzhou, China). Recombinant SARS-CoV-2-NP protein expressed in insect cells was offered by Asbio Technology, Inc. (Guangzhou, China).

The RAb was prepared by Sino Biological, Inc. (Beijing, China), which was expressed in HEK293 cells and purified with protein A affinity chromatography.

2.2. Indirect enzyme-linked immunosorbent assay (ELISA)

96-well Nunc MaxiSorp™ flat-bottom ELISA plates (Nunc, Roskilde, Denmark) were coated with recombinant proteins (1 μg/mL) or synthesized peptides (4 μg/mL) overnight at 4 °C in carbonate-buffered saline (pH 9.6). Subsequently, the plates were washed one time with 0.05 % Tween-20 in phosphate-buffered saline (PBST) and then blocked for 2 h with 2% bovine serum albumin (BSA) in PBST. RAb was added to the respective well at serial dilutions and then incubated at 37 °C for 1 h. After being washed four times with PBST, the plates underwent the 1 h incubation with a 1:10,000 dilution of horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG secondary antibody (Boster Biotech, Wuhan, China) at 37 °C. After the plates were washed four times with PBST, tetramethylbenzidine (TMB) substrate was introduced into the plates to visualize the products. The reaction was stopped with 2 M H_2SO_4, and the absorbance values were determined immediately at 450 nm with an ELISA plate reader (Multiskan MK3; Thermo Scientific)

2.3. Peptide competition ELISA

Competitive inhibition ELISA was performed to confirm the epitope detected by RAb. Optimized concentrations of the RAb were determined by conducting the serial dilution. 96-well ELISA plates were coated with recombinant NP protein in carbonate–bicarbonate buffer (pH 9.6) overnight at 4 °C and then blocked with the blocking buffer for 2 h at 37 °C. In separate tubes, peptide under increasing concentrations (0.5, 1, 2, 5, 50 or 100 μg/mL) were added to diluted RAb with the optimized concentration in the blocking buffer, and then the incubation was conducted for 1 h at 37 °C. RAb without peptide (0 μg/mL) was classified as the control. Next, each of the RAb-peptide mixtures was added to the coating plate and received the incubation for 1 h at 37 °C. The subsequent procedures were performed by complying with the instructions for the indirect ELISA.

2.4. Western blot analysis

Protein samples were mixed with 5 × loading buffer (i.e., 10 % SDS, 5% 2-mercaptoethanol, 0.5 % bromophenol blue, as well as 50 % glycerol in 250 mM Tris−HCl [pH 6.8]) and then heated for 5 min at 98 °C (denatured). Next, the samples were separated by 10 % SDS-PAGE and then electrophoretically transferred onto nitro-cellulose membranes with a wet blotter. Subsequently, the membranes were blocked with 5% skim milk in PBS and then probed with RAb at a final dilution of 1:5,000. Afterwards, the membranes were washed again and further exposed to HRP-conjugated secondary antibody. Furthermore, the blots were developed with 1-Step Ultra TMB Blotting Solution substrate (Thermo Scientific, Rockford, IL, USA) at ambient temperature.

2.5. Immunocytochemistry analysis

To verify whether RAb is available for detecting natural NP expressed in human cells, this study performed the immunocytochemistry test. In brief, the Ad293 cells in 24-well plates were transfected with plasmid pcDNA-SARS-CoV-2-NP and then received the 2-day culture at 37 °C. The plates were rinsed once with PBS and then fixed with methanol for 5 min in a −20 °C freezer. After being blocked with the blocking buffer, goat sera (5%) in PBST, for 1 h under the gentle rocking, the cells were incubated with RAb at a final dilution of 1:1000 in the blocking buffer for 1 h at 37 °C. Mouse anti−OC43-RBD and anti-PBS

Table 1
Recombinant proteins expressed in *E.coli.*

Peptide	Vector	Position[a]	Primer	Primer sequence (5′–3′)[b]
SARS-CoV-1-N	pQE30	1−422	SARS-CoV-1-N-F	GCGTGGATCCTCTGATAATGGACCCCAATC
			SARS-CoV-1-N-R	CCCAAGCTTATGCCTGAGTTGAATCAGC
SARS-CoV-2-N	pQE30	1−419	SARS-CoV-2-N-F	GCGTGGATCCTCTGATAATGGACCCCAA
			SARS-CoV-2-N-R	CCCAAGCTTAGGCCTGAGTTGAGTCAGC
OC43-N	pQE30	1−448	OC43-N-F	CGCGGATCCTCTTTTACTCCTGGTAAG
			OC43-N-R	CCCAAGCTTTTATATTTCTGAGGTGTC
nCoV-N1	pGEX-4T-3	1−120	nCoV-N1-F	GTTCCGCGTGGATCCATGTCTGATAATGGACCC
			nCoV-N1-R	TCACGATGCGGCCGCTCCAGCTTCTGGCCCAGT
nCoV-N2	pGEX-4T-3	106−225	nCoV-N2-F	GTTCCGCGTGGATCCCCAAGATGGTATTTCTAC
			nCoV-N2-R	TCACGATGCGGCCGCGTCAAGCAGCAGCAAA
nCoV-N3	pGEX-4T-3	211−330	nCoV-N3-F	GTTCCGCGTGGATCCGCTGGCAATGGCGGTGAT
			nCoV-N3-R	TCACGATGCGGCCGCCCACGTTCCCGAAGGTG
nCoV-N4	pGEX-4T-3	316−419	nCoV-N4-F	GTTCCGCGTGGATCCGGAATGTCGCGCATTGGC
			nCoV-N4-R	TCACGATGCGGCCGCGGCCTGAGTTGAGTCAGC

[a] Numbers show the location of the peptide in NP protein.
[b] Restriction sites are underlined in the primer pairs used for cloning.

sera were added to other wells as the controls. After being washed, the cells were diluted at 1:2000 to HRP-conjugated goat anti-mouse secondary antibody. After washing with PBST, the cells were developed with 1-Step Ultra TMB Blotting Solution substrate (Thermo Scientific, Rockford, IL, USA) and observed under the microscope.

The HCT-8 cells were obtained from ATCC (CCL-244) and maintained in 1640 medium (Gibco, USA) supplemented with 100 IU/mL penicillin, 100 mg/mL streptomycin, 1% Non-essential amino acids, 25 mM Hepes, as well as 10 % fetal bovine serum. HRT-8 cells were infected with 0.01 MOI OC43 coronavirus obtained from ATCC (VR-1558) in 24-well plates and then received the 4-day culture at 33 °C in a humidified 5% CO_2 atmosphere. Next, the plates were treated and observed as above. Mouse anti−OC43-RBD and anti-PBS sera were used as the controls.

2.6. Double-antibody sandwich ELISA

Double-antibody sandwich ELISA was established to further comment this RAb as a successful tool for diagnostics. In briefly, ELISA plates were coated with RAb (2 μg/mL) in PBS (pH 7.4) overnight at 4 °C and then blocked with the blocking buffer for 2 h at 37 °C. Purified SARS-CoV-2-NP protein expressed in insect cells under increasing concentrations (0, 0.0064, 0.032, 0.16, 0.8, 4, 20 μg/mL) were added to the respective well in the blocking buffer, and then incubated at 37 °C for 1 h. After being washed four times with PBST, mouse antiserum against SARS-CoV-2 NP (1:2,000 dilution) was added to the plate and received the incubation for 1 h at 37 °C. Then the plates were incubated with a 1:10,000 HRP-conjugated goat anti-mouse IgG secondary antibody, and then developed with TMB substrate. After stopped the absorbance values were determined. The standard curve was then drawn. Ad293

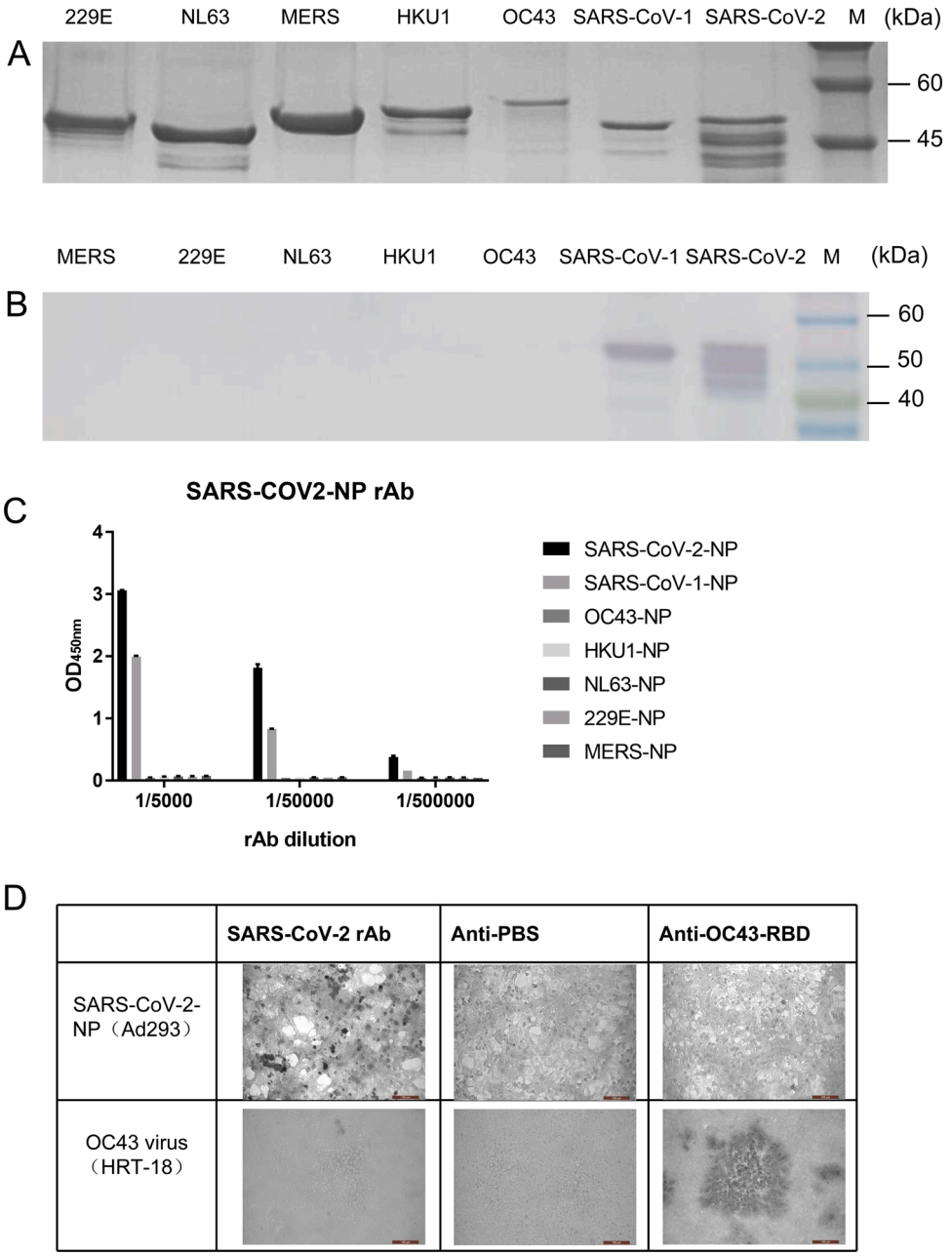

Fig. 1. Characterization of RAb recognizing human CoV NPs. (A) Coomassie-stained SDS-PAGE of purified NPs of seven human CoVs. (B) and (C) Western blot and ELISA analysis of RAb cross-reacting with NPs of seven human CoVs. M: protein ladder marker. (D) Immunocytochemistry analysis of RAb detecting Ad293 cells transfected with pcDNA-SARS-CoV-2-NP, and HRT-8 cells infected with CoV OC43. Mouse anti-OC43-RBD and anti-PBS sera were used as the controls.

cells were transfected with pcDNA-SARS-CoV-2-NP and then collected in PBS after receiving 2-day culture. The cells after freeze-thaw were centrifugated to collect supernatant which contained SARS-CoV-2 NP protein. The SARS-CoV-2 NP protein in cellular lysis samples could be quantified by the established double-antibody sandwich ELISA. The lysis supernatant of Ad293 cells transfected with pcDNA-3.1 were diluted with PBS to 10 μg/mL and used as the control. The cultured virus samples, human adenovirus type 7 (HAdV-7) GZ08 strain (Genebank No. GQ478341.1), human respiratory syncytial virus A strain Long (RSV) from ATCC (Genebank No. AY911262.1), influenza virus A strain PR8 (A/PR8/34, H1N1), HCoV−OC43 strain VR-1558 from ATCC, HCoV-229E strain VR-740 from ATCC, which were maintained at our laboratory, were also added as the controls.

3. Results

3.1. Identification and cross-reaction of RAb with human CoV NPs

Seven recombinant coronavirus NPs of SARS-CoV-1, SARS-CoV-2 and OC43 were expressed and purified in *Escherichia coli* to identify the specificity of RAb and the cross reaction SARS-CoV-1 and SARS-CoV-2 (Fig. 1A). As demonstrated from the results of the western blotting assay, RAb specifically recognized both NPs of SARS-CoV-1 and SARS-CoV-2, but not the other human coronavirus NPs (Fig. 1B), as confirmed by indirect-ELISA (Fig. 1C). Moreover, human cells transfected with pcDNA-SARS-CoV-2-NP could be detected by RAb by performing the immunocytochemistry (Fig. 1D). As opposed to the mentioned, the cells transfected with pcDNA-SARS-CoV-2-NP could not be detected with the control mouse antisera, anti-PBS and anti−OC43-RBD. HRT-8 cells infected with OC43 coronavirus could not be detected by RAb and mouse anti-PBS sera, but it could be detected by mouse anti−OC43-RBD (Fig. 1D). Thus, RAb recognized the common epitope shared by SARS-CoV-1 and SARS-CoV-2, which is considered continuous based on the reactivity with the denatured protein.

3.2. Mapping and characterization of epitope detected by RAb

To identify the region of SARS-CoV-2 NP bound with RAb, four truncated recombinant proteins with GST tag were expressed in Escherichia coli, and 15 amino acids were found overlapped in the adjacent truncated proteins (Fig. 2A). As revealed from the results of SDS-PAGE, four truncated recombinant proteins were successfully expressed and then purified (Fig. 2B). According to the results of the indirect-ELISA and the western blotting assays, RAb reacted only with N4-GST (Fig. 2C and 2D), suggesting that RAb recognized epitope in the N4 region of SARS-CoV-2 NP.

To identify the epitope recognized by RAb in depth, 9 short peptides overlapping the N4 region of SARS-CoV-2 were synthesized (Fig. 3A). Indirect-ELISA demonstrated that RAb only reacted with SARS−COV-2-N4−8 (Fig. 3B). Further peptide competition ELISA was performed to verify whether the identified epitope for RAb was accurate. Peptide SARS−COV-2-N4−8 inhibited RAb binding with SARS-CoV-2 NP in a dose-dependent manner, whereas another peptide did not (Fig. 3C). Thus, N4−8 was the epitope recognized by RAb.

As demonstrated from the alignment of the NP sequences of SARS-CoV-2 available from Genbank, the epitope for RAb was currently conserved in all global SARS-CoV-2 strains, while there was no substitution in the region (data not shown). NP phylogenetic tree was built with the Neighbor-joining method (Fig. 4A), with five representatives of the species SARSr-CoV, and other five human CoVs. SARSr-CoV of the genus β-coronavirus was the most closely related. SARSr-CoV BtKY72 strain was relatively far from other SARSr-CoV strains (SARS-CoV-1, SARS-CoV-2, SARSr-CoV RaTG13) which cluster a branch. To gain insights into the distribution of the epitope SARS-CoV-2-N4−8 among different human coronaviruses, the NP protein sequences were aligned. The epitope SARS-CoV-2-N4−8 was common for SARS-CoV-1 and SARS-CoV-2, whereas it was unique to other human coronaviruses (e.g., α-coronavirus (HCoV-229E, HCoV-NL63) and β-coronavirus (HCoV−OC43, HCoV-HKU1, MERS-CoV)) (Fig. 4B). It is noteworthy that this SARS-CoV-2-N4−8 epitope was highly conserved in most SARSr-CoVs (e.g., bat CoVs SARSr-CoV-RaTG13 and SARSr-CoV-BtKY72) closed to SARS-CoV-1 and SARS-CoV-2(Fig. 4C). There was an amino acid in corresponding epitope of SARSr-CoV BtKY72 strain isolated from bat, which was not consistent with other SARSr-CoVs (D399E). We also synthesized this peptide of BtKY72 strain, and found it could not be detected by the RAb (Fig. 4D). This result demonstrated that D399 may be a critical amino acid for RAb binding.

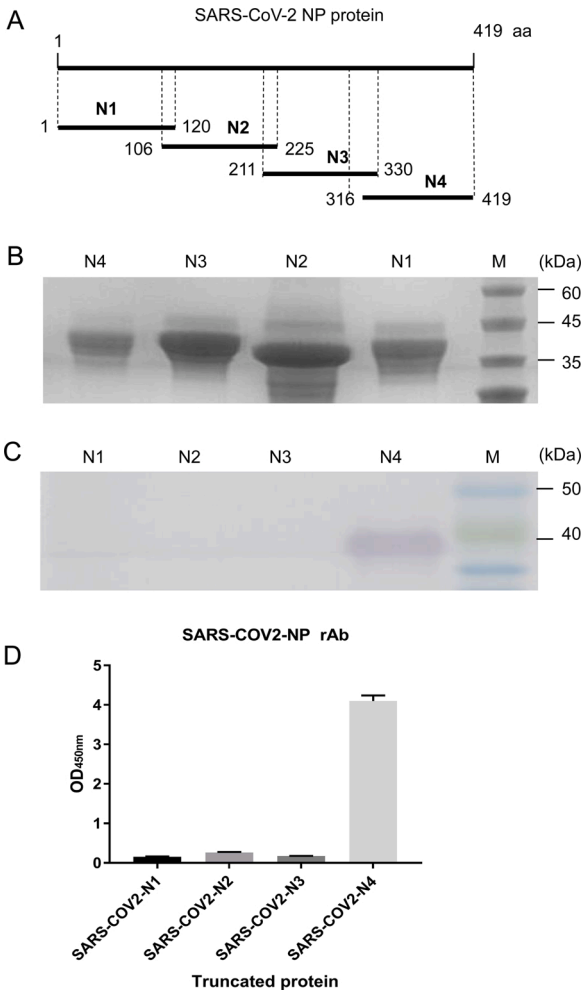

Fig. 2. Mapping of RAb with truncated NP fragments. (A) Schematic diagram of truncated peptides of SARS-CoV-2 NP. (B) Coomassie-stained SDS-PAGE of four purified truncated fragments of SARS-CoV-2 NP. M: protein ladder marker. Western blot (C) and ELISA (D) analysis of RAb with four truncated fragments indicated the c-terminal peptide N4 was the target of RAb.

3.3. Quantification of SARS-CoV-2 NP by double-antibody sandwich ELISA

The double-antibody sandwich ELISA was established using purified SARS-CoV-2-NP expressed in eukaryotic cells as the standards (Fig. 5A). The protein could be well quantified under concentrations from 0.032 to 4 μg/mL. By this method the SARS-CoV-2-NP expressed in Ad293 cells was quantified, which was about 0.19 μg/mL. Importantly, the cultured virus samples of HAdV-7, RSV, InfA, HCoV−OC43 and HCoV-229E could not be detected by this sandwich ELISA (Fig. 5B). This result suggested a high specificity of this sandwich ELISA method.

Accordingly, RAb was revealed as a very useful tool to distinguish the

A

Peptide	Amino acid sequences	Amino acid position
SARS-COV2-N4-1	GMSRIGMEVTPSGT	316-329
SARS-COV2-N4-2	SGTWLTYTGAIKLD	317-340
SARS-COV2-N4-3	KLDDKDPNFKDQVI	338-351
SARS-COV2-N4-4	QVILLNKHIDAYKT	349-362
SARS-COV2-N4-5	YKTFPPTEPKKDKK	360-373
SARS-COV2-N4-6	DKKKKADETQALPQ	371-384
SARS-COV2-N4-7	LPQRQKKQQTVTLL	382-395
SARS-COV2-N4-8	TLLPAADLDDFSKQL	393-407
SARS-COV2-N4-9	KQLQQSMSSADSTQA	405-419

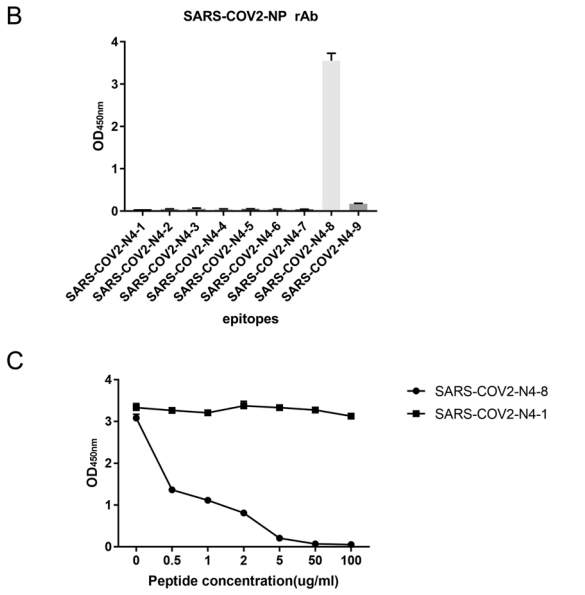

Fig. 3. Epitope mapping of RAb with synthetic peptides. (A) Nine synthetic peptides of N4 fragment of SARS-COV-2 NP. (B) ELISA to map the epitope recognized by RAb with synthetic peptides. (C) Competition ELISA to confirm the epitope recognized by RAb. RAb under constant concentration was pre-incubated with increasing concentrations of the peptide SARS-COV-2-N4-8 or the control peptide SARS-COV-2-N4-1, and then added to the plate coated with SARS-COV-2 NP.

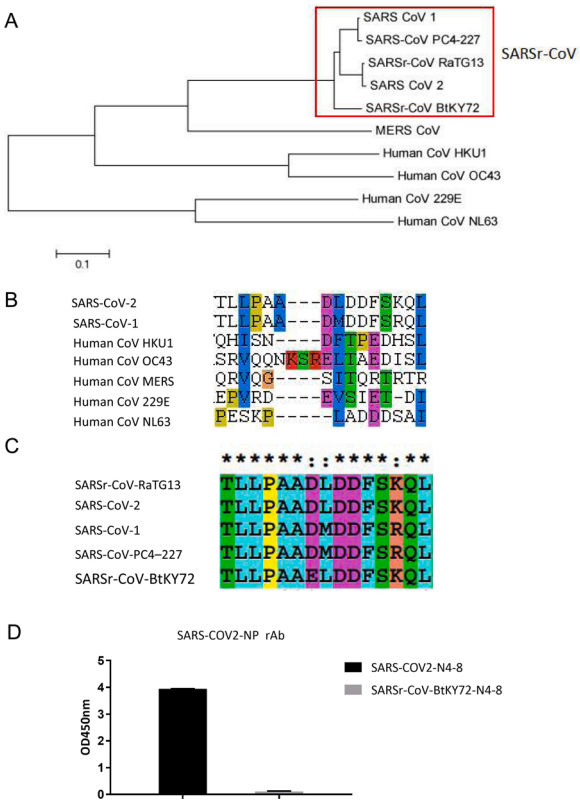

Fig. 4. The N4-8 epitope mutation analysis in human CoVs and SARSr-CoVs. (A) The Neighbor-joining tree of NPs from five SARSr-CoVs and five human CoVs. (B) Sequence alignment of the N4-8 epitope of seven human CoVs. (C) Sequence alignment of the N4-8 epitope of five SARSr-CoVs. (D) N4-8 peptide from SARSr-CoV-BtKY72 was synthesized and analysed with RAb by ELISA, showing no reaction with RAb.

SARS-CoV-2 and SARSr-CoVs from other common human CoVs, and may help identify novel members of SARSr-CoVs.

4. Discussion

Thus far, the 2019 novel coronavirus still affects a considerable number of nations. Developing a rapid and accurate diagnostic method is of critical significance for curbing the prevalence of SARS-CoV-2. The N protein sequence of coronavirus shows the smallest variation in genome, so it has been extensively applied for diagnosing the novel coronavirus. SARS-CoV-1 is the most consistent with SARS-CoV-2. The N protein of SARS-CoV-1 exhibits a dimer structure, containing two independent folding domains, termed as N-terminal domain (NTD) and C-terminal domain (CTD) (Chang et al., 2014, 2006). The middle is connected by linker region (LKR), and the two ends are commonly composed of two internally ordered regions (IDR) (Chang et al., 2014, 2009; Peng et al., 2008). Moreover, the RNA binding activity of N protein is crucial for viral RNP and genome replication, capable of leading to humoral and cellular immune responses after infection (Ni et al., 2020; Xiang et al., 2020). For the mentioned reason, N protein refers to one of the targets for diagnosis and vaccine design.

The epitope information of N protein helps develop epitope based diagnostic reagents and vaccine design. In this experiment, the specificity of rabbit monoclonal antibody and the B cell epitope of N protein were identified. In the epitope mapping analysis, TLLPAADLDDFSKQL was the rabbit monoclonal antibody recognition site (Fig. 3B), located in the C-tail of N protein. In the N-CTD crystal structure diagram (Chang et al., 2014), the epitope of C-tail was exposed on the surface (Peng et al., 2020). Existing studies were conducted on N protein as a drug target (Lin et al., 2014; Zhang et al., 2020). However, the study of C-tail site has not been reported before, so this rabbit monoclonal antibody can also be exploited to study the structure and function of N protein C-tail. The RAb could detect the synthesized peptide, and the epitope on SARS-CoV-2 NP proteins from *E.coli*, insect cells, and human cells. In addition, RAb could detect natural SARS-CoV-2 NP by immunocytochemistry analysis and ELISA, also denatured SARS-CoV-2 NP by western blot analysis. These results demonstrate that N4−8 epitope detected by the RAb is a linear epitope exposed on the NP protein surface.

Though N protein is similar to other α-coronaviruses in structure and function, this epitope located in C-tail does not cover any obvious sequence homology (Fig. 4B). In the experiment of this study, the used rabbit monoclonal antibody could cross-recognize SARS-CoV-1 and SARS-CoV-2, whereas it failed to recognize other common human coronaviruses (OC43, NL63, HKU1 and 229E), demonstrating that the specificity of RAb was high and the RAb could be used to develop a diagnosis method for distinguishing SARS-CoV-2 from common human CoVs. In addition, this study attempted to detect the epitopes of other SARSr-CoVs (e.g., SARS-CoV-PC4−227, SARSr-CoV-RaTG13 and SARSr-CoV-BtKY72) in SARS-CoV-2-N4−8 by employing rabbit monoclonal antibody, whereas there was an amino acid in SARSr-CoV-BtKY72, which was not consistent with other SARS related CoVs. As revealed from the results of this study, this amino acid could affect the binding of rabbit monoclonal antibody (Fig. 4D), demonstrating that it is the key

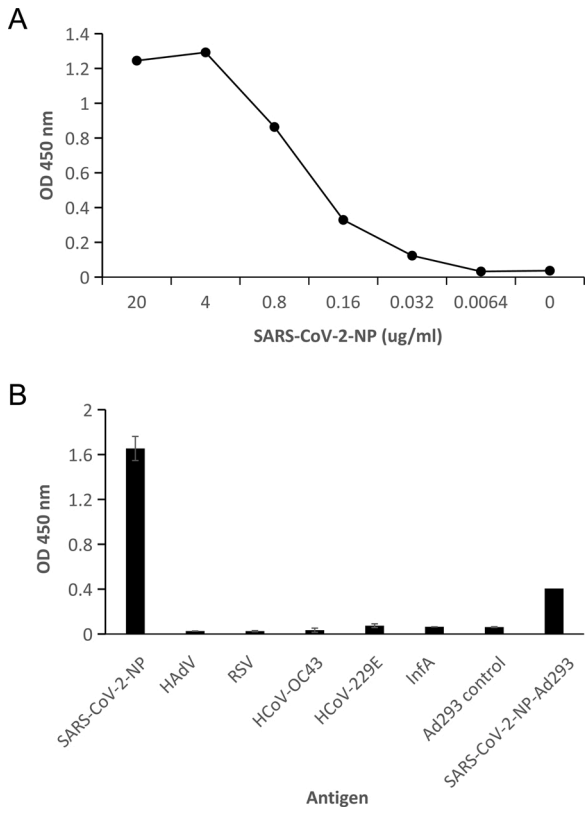

Fig. 5. Quantification of SARS-CoV-2 NP by double-antibody sandwich ELISA. (A) Double-antibody sandwich ELISA was established. The plates were coated with RAb, then incubated with purified SARS-CoV-2-NP protein under increasing concentrations. Mouse antiserum against SARS-CoV-2-NP and HRP-conjugated secondary antibody was then added in turn. (B) Double-antibody sandwich ELISA was used to quantify SARS-CoV-2-NP expressed in Ad293 cells. Purified SARS-CoV-2-NP (2 μg/mL) was used as the positive control, and Ad293 cellular supernatant was used as the negative control. HAdV-7, RSV, InfA, HCoV-OC43, and HCoV-229E could not be detected by this sandwich ELISA.

amino acid (D399) for antibody binding.The mentioned data will theoretically underpin the development of epitope based diagnostic methods and vaccine research.

Here we established a sandwich ELISA to quantify SARS-CoV-2-NP, which may be used for SARS-CoV-2 vaccine quantification, or clinical SARS-CoV-2 detection. This method proved the high specificity of RAb detecting SARS-CoV-2-NP (Fig. 5B), and provided an example for the RAb as a tool for diagnostics. However, the RAb should be further evaluated as a successful tool for diagnostics with wild SARS-CoV-2 strains and clinical SARS-CoV-2 samples in the future work.

In brief, the rabbit monoclonal antibody is capable of distinguish SARS−COV-1 and SARS−COV-2 from common human CoVs, which can act as a tool for auxiliary diagnosis and detection. The RAb can recognize the four SARSr−COVs, other than SARSr−COV-BtKY72, and may also identify novel SARSr−COVs in the future, which shows promising high clinical applications.

Author statement

Authors' individual contributions: XT planned and designed the study; XT and CM wrote the manuscript; XT, CM, ZZ, LZ, YY, AY, YF, WL, and XL performed the experiments; XT and RZ guided the study. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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