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Journal of Veterinary Medicine and Research

Research Article

The Combined Effect of a Novel Vaccine for Herd Immunization on PRRSV and PCV on Jinzhong-Shanxi of China

Longlong Zheng, Fan Tan, Huixin Kang, Wanghua Liu, Chen Wang and Li Zhang*

College of Veterinary Medicine, Shanxi Agricultural University, China

***Corresponding author**

Li Zhang, College of Veterinary Medicine, Shanxi Agricultural University, Taigu, Shanxi 030801, China

Submitted: 20 October 2022

Accepted: 25 November 2022

Published: 29 November 2022

ISSN: 2379-948X

Keywords

- *PRRSV inactivated vaccine; NADC30-like; PCV2 VLPs vaccine; PCV3*

Abstract

PRRSV and PCV were two of the most significant swine diseases that affect the pig industry. NADC30-like or NADC34-like PRRSV and PCV3 have had a strong in China and could cause clinical disease on pigs. Co-infection of PRRSV and PCV is frequently observed under field conditions and elicits more severe diseases. However, the endemic status of NADC30-like/NADC34-like PRRSV and PCV3/PCV4 in Jinzhong of Shanxi is unclear under the field condition of immune inactivated vaccines or subunit vaccine (Virus-LikeParticles, VLPs). In this study, we examined 120 weaner dead pigs-lung tissue samples collected from a single PRRSV and PCV positive pig farm in Jinzhong-Shanxi from January to November 2021. Six NADC30-like PRRSV and PCV3 strains were discovered in samples, and PRRSV NSP2 and ORF5 genomes & PCV3 Cap genomes of these strains were sequenced. Phylogenetic analysis indicated that these novel PRRSV and PCV strains belong to NADC30-like PRRSV and PCV3, forming one group in Shanxi, Jinzhong. After NADC30-like PRRSV, pregnant sows (African swine fever infection pressure) were immunized with Ch-1a inactivated vaccine for one year, NADC34-like PRRSV was not identified, and NADC30-like PRRSV was still an epidemic strain in the piglet herd. Importantly, PCV2 pregnant sows were immunized with subunit (Cap protein) vaccine for one year, PCV-4 was not detected, and PCV-3 was still an epidemic strain in the piglet herd. Immunization of sows with PRRSV inactivated vaccine and circular VLPs vaccine can reduce viremia and increase the survival rate of piglets, but cannot provide complete virological protection.

ABBREVIATIONS

PCV: Porcine circovirus; PRRSVs: Porcine Reproductive and Respiratory Syndrome Viruses; PRRSV: Porcine Respiratory and Reproductive Syndrome Virus; PCV1: Porcine Circovirus Type 1; PCV2: Porcine Circovirus Type 2; PCV3: Porcine Circovirus Type 3; PCR: Polymerase Chain Reaction; nt: Nucleotides; PNDS: Porcine Dermatitis and Nephropathy Syndrome; ORF: Open Reading Frame; Rep: Replication-associated protein; Cap: Capsid; VLPs: Virus-Like Particles.

INTRODUCTION

Co-infection of PRRSV and PCV was commonly observed under field conditions and elicits more severe diseases. The co-infection of PRRSV and PCV (PCV2 and PCV3) might cause diseases even when PRRSV plays a limited role in the pathogenicity of the co-infection [1]. PRRSV can be regulated to a certain extent with macrolide drugs (Tilmicosin and Tyvanectin). PCV3 is associated with several clinical signs called porcine circovirus-associated diseases (PCVAD). There are still no effective drugs to prevent and control PCV, they can cause clinical diseases alone, and the economic loss is getting higher and higher. Vaccination is the best way to avoid economic losses from PRRSV and PCV. In China, although various commercial attenuated PRRSV-live vaccines, PRRSV and PCV-inactivated vaccines and PCV-subunit vaccines have been widely utilized, PRRS and PCV are still severe in the pig industry.

The modified-live virus (MLV) vaccines provide extremely limited cross-protection efficacy against the NADC30-like virus CHsx1401 infection [2], the NADC30-like strain HNhx [3]. Also, the inactivated vaccines revealed partial protection hp-PRRSV QH-08 Strain [4], and maternal inactivated vaccines vaccination can improve protection of pre-weaning piglets against PRRSV that transferring neutralizing antibodies to piglets [5]. As a result, maternal-derived immunity is a critical component for the survival and success of offspring in pigs to protect from circulating pathogens such as PRRSV and PCV.

Moreover, the genetic diversity and complexity of PRRSV were further increasing. Constructed the global classification system of PRRSV based on the comprehensive analysis of the complete ORF5 gene sequence [6]. Since 2013, PRRS became prevalent again in China caused by new PRRSV variants, NADC34-like strains, which are considered to be imported from North American and adapted in China. NADC30-like or NADC34-like PRRSV virus strains transmitted quickly around herds in China although massive vaccination with all the commercial vaccines. Animal challenge study showed that the novel NADC30-like PRRSV SD17-36 isolate is low pathogenic [1], and CHsx1401 [7], & HB17A [8], exhibits intermediate virulence.

Therefore, this study aimed to assess the positivity rates of PCV3/PCV4 and NADC30-Like/ NADC34-Like PRRSV in a large-scale pig farm in Jinzhong-Shanxi Province, under immunity PRRSV CH1a and PCV2 VLPs vaccines during 1 year period.

MATERIALS AND METHODS

Sample collection

Lung tissue samples (n = 120; 1.5-2 month-old pigs) were collected from one pig farm was major large-scale farm in Shanxi Province, which was distributed in Jinzhong city of Shanxi Province, accounting for about 3% of the total nursery stage. The samples were collected between January and November in 2021. Lung tissues were randomly selected from the respiratory syndrome clinical symptoms and anatomical dead, put into a sterile bag containing tissue protection solution, placed in an icebox, transported back to the laboratory for RT-PCR or PCR and sequencing analysis.

RNA/DNA isolation and polymerase chain reaction (PCR)

RNA/DNA were extracted from the collected tissue samples using the TRIzol®(TaKaRa, Dalian, China) and the EasyPure® Genomic DNA Kit (TransGen Biotech, Beijing, China) according to the manufacturer's instructions. cDNA was generated using a PrimeScriptTM RT reagent Kit (TaKaRa, Dalian, China) following the supplier's guidelines. PCV3 with PRRSV were detected using reverse transcription (RT)-PCR and PCR. The primers (PRRSV-F: 5'-TTGATTGGGATGTTGTGCTTC-3', PRRSV-R: 5'-CAATGATGGCTTGAGCTGAGT-3') were designed based on the NSP2 region, and the sizes of the final amplicons were 628 bp (NADC30-like PRRSV), 931 bp (HP-PRRSV), and 1,021 bp (typical PRRSV). This yielded a 603-bp PCR product containing a complete ORF5 gene of PRRSV [9]. The PCV3 specific primers designated as PCV3-F: 5′-TAGTATTACCCGGCACCTCGGAACC–3′, and PCV3-R: 5′- ACAGGTAAACGCCCTCGCATGTGGG–3′, which amplified 649-bp [10]. The cycling conditions were: 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 57°C for 30 s, 72°C for 35 s, and a final extension at 72°C for 7 min.

The PCR products were analysed using gel electrophoresis on a 1.5% agarose gel in Tris-acetic-acid-EDTA buffer and stained with Ethidium Bromide (Ruitaibio, Beijing, China). The genome of the six of NADC30-like PRRSV and PCV3 strains (in this study) were registered in National Microbiology Data Center (NMDC) No. shown Table 1.

Genome sequencing

Further, the purified RT-PCR or PCR products were sequenced by Sangon Biological Technology (Beijing, China).

Genome alignment and phylogenetic analysis

The obtained full-length genomic sequences were assembled using the SeqMan program of DNAstar software, version 7.0 (DNASTAR Inc., Madison, WI, USA). Further, the sequences of PRRSV NSP2/ORF5 and PCV3 Cap and deduced proteins were analyzed by the EditSeq and MegAlign programs of DNAstar (DNASTAR Inc., Madison, WI, USA). Next, phylogenetic analysis was performed based on the nucleotide sequences, generated by the distance-based neighbor-joining method using the Molecular Evolutionary Genetics Analysis 6 (MEGA 6) software (www.megasoftware.net). Finally, the bootstrap values of the phylogenetic tree were evaluated with 1000 replicates. PRRSV and PCV strains used in our study of phylogenetic trees shown Table 2,3.

Statistical analysis

The statistical significance of the differences was assessed by Student's t test and one-way ANOVA with multiple comparisons. Significant differences in the survival curves were determined by log-rank analysis. A P value less than 0.05 was considered to indicate statistical significance, P < 0.01 was considered highly significant, P<0.001 was considered very highly significant, and P<0.0001 was considered extremely highly significant.

Table 1: Detection of NADC30-like PRRSV and PCV3 strains from Jinzhong-Shanxi in January. 2021 to November. 2021.

Name of sample/ Specimens	Clinical symptoms	Pig group*	Collection date	NMDC No.		
				PRRSV NSP2	PRRSV ORF5	PCV3
SXJZ-2021-01/Lung	Persistent diarrhea, Wasting, Papular dermatitis, Respiratory disorders, Hind Limb Paralysis, Death	Weaner	10.January.2021	NMDCN0000R26	NMDCN0000R2E	NMDCN0000R5U
SXJZ-2021-02/Lung	Intermittent diarrhea, Wasting, Papular dermatitis ,Respiratory disorders, Hind Limb Paralysis, Death	Weaner	12.March.2021	NMDCN0000R27	NMDCN0000R2D	NMDCN0000R5V
SXJZ-2021-03/Lung	Intermittent diarrhea, Wasting, Respiratory disorders, Hind Limb Paralysis, Death	Weaner	11.May.2021	NMDCN0000R28	NMDCN0000R2C	NMDCN0000R60
SXJZ-2021-04/Lung	Intermittent diarrhea, Wasting, Respiratory disorders, Hind Limb Paralysis, Death	Weaner	13.July.2021	NMDCN0000R29	NMDCN0000R2B	NMDCN0000R64
SXJZ-2021-05/Lung	Intermittent diarrhea, Wasting, Respiratory disorders, Hind Limb Paralysis, Death	Weaner	10.September.2021	NMDCN0000R2G	NMDCN0000R2A	NMDCN0000R65
SXJZ-2021-06/Lung	Intermittent diarrhea, Wasting, Respiratory disorders, Hind Limb Paralysis, Death	Weaner	12.November.2021	NMDCN0000R2F	NMDCN0000R29	NMDCN0000R67

*Samples were classified into six groups of time, suckling pigs (<30 days), weaner (30–60 days), grower (60–90 days) and finisher (≥90 days)

Table 2: PRRSV strains used in our study of phylogenetic trees.

No.	Strain/ Genotype	Country	Year	GenBank No.
1	CH-1a/Typical	China	1996	AY032626
2	VR2332/Typical	USA	2002	AF535152
3	R98/Typical	China	2006	DQ355796
4	CH-1R/Typical	China	2008	EU807840
5	WUH6/Typical	China	2016	KU523367
6	JXA1-R/HP-PRRSV	China	2020	MT163314
7	TJM-F92/HP-PRRSV	China	2020	MN508255
8	HUN4/HP-PRRSV	China	2016	EF635006
9	GDr180/HP-PRRSV	China	2018	MG972942
10	SD2017/NADC30-link	China	2017	MH500776
11	NADC30-USA-2012	USA	2012	JN654459
12	CHsx1401/NADC30-link	China	2014	KP861625
13	SD17-36/NADC30-link	China	2018	MH121061
14	Guangdong/NADC34-link	China	2017	LNWK96
15	Guangdong/NADC34-link	China	2017	LNWK130
16	HNLCL82-1811/NADC34-link	China	2018	MN648057
17	RFLP 1-4-4	USA	2020	MW887655
18	P129/Typical	USA	2002	AF494042
19	GM2	China	2011	JN662424
20	SX2009	China	2009	FJ895329

Table 3: PCV strains used in our study of phylogenetic trees.

No.	Strain/ Genotype	Country	Year	Accession
1	PCV1	China	2015	KP337349
2	PCV1-Qu	Canada	2021	MK872393
3	ZJ/C/PCV2	China	2004	AY686764
4	DBN-SX01/PCV2	China	2006	GQ404800
5	SH/PCV2	China	2006	HM038027
6	DBN-SX07-2/PCV2	China	2007	HM641752
7	LG/PCV2	China	2008	HM038034
8	SXJZ/PCV2	China	2014	KX068221
9	14SX01/PCV2	China	2015	KP975432
10	WH/PCV2	China	2015	MK604497
11	CN-Shandong-2-201703/PCV3	China	2017	KY778777
12	CN-HLJ-p6-2018/PCV3	China	2018	MN431643
13	Pig-CN-HeNan170546-3/PCV3	China	2018	MF769809
14	Shanxi-2018/PCV3	China	2018	MH548436
15	SDA004-2018/PCV3	China	2018	MK178296
16	CN-Xinjiang-AL15/PCV3	China	2018	MK562413
17	Henan-2019/PCV4	China	2019	MT002818
18	ZJ-459-20130424/PCV3	China	2019	MK744559
19	Shanxi-2020/PCV3	China	2020	MZ449243
20	GX2020/ PCV4	China	2020	MT311854

RESULTS

Changes in Herd Immunization Vaccines (Table 4)

After long-term whole herd immunization with PRRSV inactivated vaccine and PCV2 VLPs vaccine, the mortality rate of pigs in the breeding period (12%~5.5%), PCVAD (15%~8%), PRRSVs PCR positive rate (45%~10%) and PCV PCR positive rate (90%~75%) showed a significant weakening trend (Figure 1).

NADC30-link NSP2 and ORF5 dataset-phylogenetic analysis

In this study, we focus only on the PRRSV strains reduction. Safer inactivated PRRSV vaccines are used in the present investigation, whole pig herd 12 months in jinzhong, Shanxi, China. Moderate virulence strains (MLV-PRRSV) NADC30-like PRRSVs gradually became dominant strains, replacing highly pathogenic strains (HP-PRRSV). NADC30-like PRRSVs are undergoing a reduction in population genetic diversity in the farm by genetic evolution analysis of NSP2 and ORF5 gene of 6 isolates.

PCV3 Capsid dataset-phylogenetic analysis

PCV2 VLPs vaccine does not prevent vertical transmission of PCV3 within the pig farm and does not provide virological protection (Figure 2)

DISCUSSION

PRRSV is highly prevalent in pig populations and is responsible for severe economic losses to the swine industry worldwide. The newly emerged lineage 1 PRRSVs (especially the NADC30-like and NADC34-like viruses) have posed a direct threat to the Chinese pig industry since 2013. Similarly, to other RNA viruses, PRRSV has the ability to continuously undergo genetic/antigenic changes [11]. PCV3 is a newly identified circovirus from swine in the USA in 2015 though metagenomic sequencing and may be associated with PDNS [12]. PCV3 may be a important pathogen in

Table 4: Vaccine impact.

Collection date	Mortality throughout the incubation period	PCVAD	PRRSVs PCR positive rate	PCV PCR positive rate
September.2020	12%	15%	45%	90%
January.2021	11.5%	15%	42%	90%
February.2021	9.5%	13%	40%	85%
Mar.2021	8.5%	13%	40%	80%
April.2021	8.5%	12%	35%	80%
May.2021	7%	12%	30%	80%
June.2021	7.5%	10%	25%	75%
July.2021	7.5%	10%	20%	70%
August.2021	7%	10%	20%	70%
September.2021	7%	9%	20%	65%
October.2021	6.2%	9%	25%	70%
November.2021	5.5%	8%	15%	70%
December.2021	5.5%	8%	10%	65%

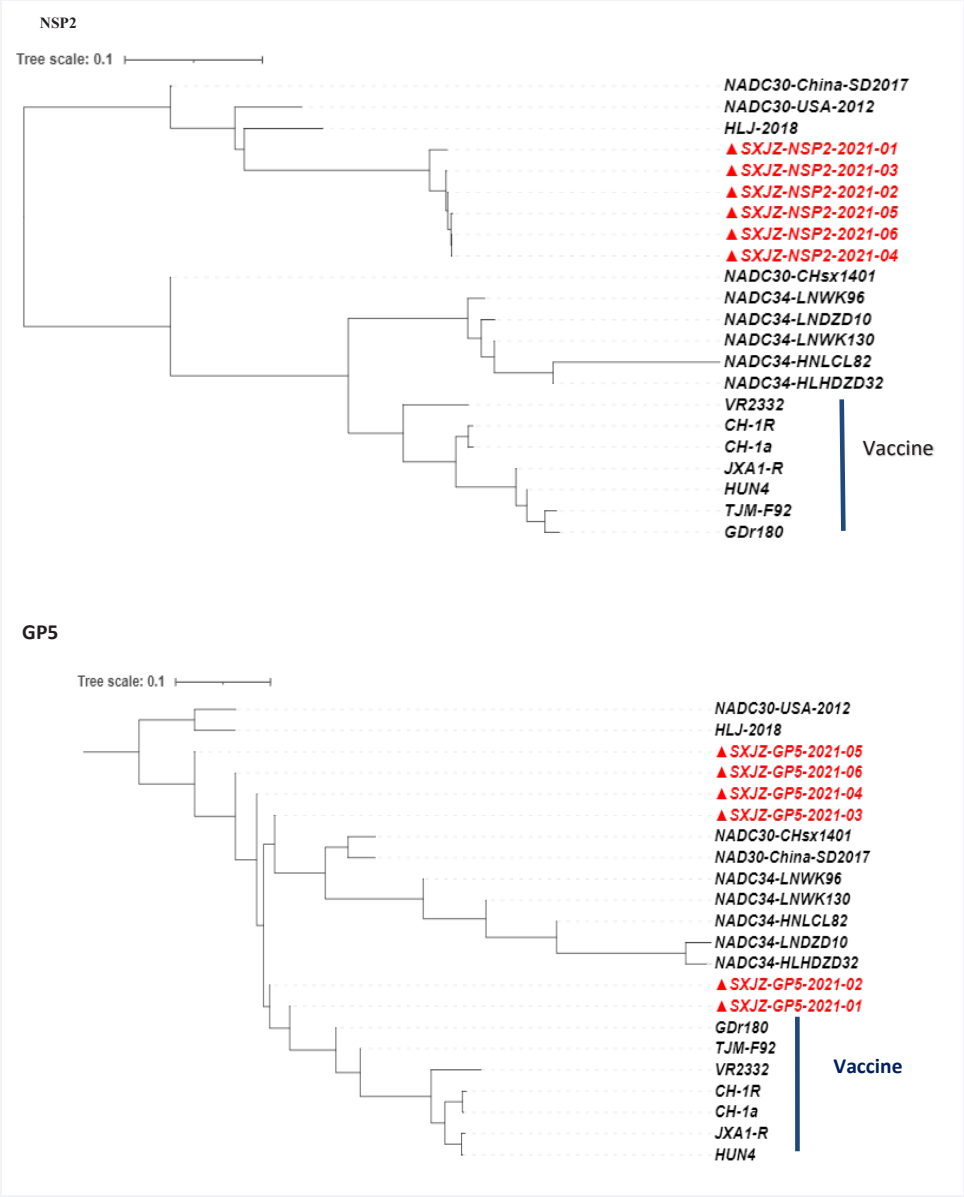

Figure 1 The genomic organization and phylogeny of NADC30-link PRRSV.

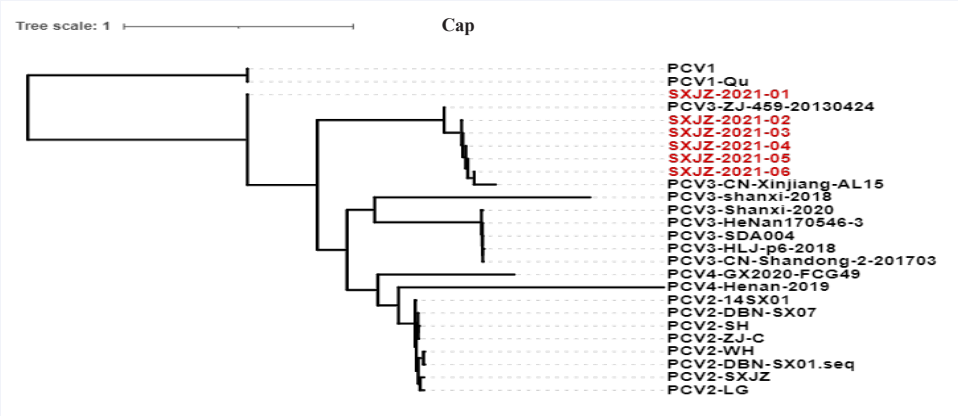

Figure 2 The genomic organization and phylogeny of PCV3 Cap.

the swine industry, infection and the disease caused by PCV3, has been reported in many swine farms worldwide with high positive rates. The PCV3 infection can lead to respiratory diseases, digestive disorders, reproductive disorders, multisystemic inflammation, and immunosuppression [13].

In China, PCV3 was first reported in Guangdong Province in 2016 [14]. The first NADC34-like PRRSV in China was reported in Liaoning province in 2018, designated LNWK96 and LNWK130 [15]. After that, 20 more mildly pathogenic NADC34-like PRRSVs and 600 PCV3 were reported in Heilongjiang, Henan, and Fujian provinces [16-20]. Only one case of NADC30-like PRRSV CHsx1401 strain has been reported in Shanxi, and few epidemiological reports on NADC34-like PRRSV and PCV3 strain has been made. Central Shanxi, mainly located in the basin area, has lush vegetation and low winds, making it an unfavorable environment for pathogen transmission [21]. The relatively stable climate is not conducive to the introduction of the virus and is an ideal place to explore the evolution of NADC34-like PRRSVs and PCV3 in pigs under immune conditions.

Vaccination is always the primary option to control and eradicate deadly diseases e.g., PRRS and PCVAD. Still, some inactivated/VLPs vaccines do not often protect the animals against PRRSV and PCV challenges when major antigenic shifts or novel virus subtypes appear. NADC30-like SXJZ-2021 strains show high genetic variations and incidence of recombination, compared with lineage 8 (MLV Ch-1a-like) and New Intro cluster (NADC30-like CHsx1401). PCV3 SXJZ-2021 strains show high genetic variations, compared with PCV2-b DBN-SX. These characteristics probably made current vaccines ineffective and confer PRRS and PCVAD much easier to escape the immune surveillance. Thus, they adapted well during the pig populations.

Vaccines are usually effective strategies for virus control. It is still a controversial question about the usage of PRRSV vaccines, achieved a certain level of clinical protection. There are no better choices currently under the circumstances of PRRSV pandemics [22]. Since the emergence of PRRSV, several different kinds of vaccines have been developed and widely adopted in the field, the most common of which are inactivated vaccines and live attenuated vaccines. However, inactivated vaccines against PRRSV cannot elicit a strong immune response, and live attenuated vaccines usually provide effective homologous protection but limited protection against heterologous strains, owing to the vast genetic diversity and high mutation rate of PRRSV [23].

But, inactivated vaccines are safer, more stable, and easier to store, higher antigen content and no ADE phenomenon compared with live vaccine. So, more and more inactivated vaccines are used in Chinese pig farms to replace live vaccines for immunization to prevent and control PRRS. These vaccines are effective in reducing clinical signs, decreasing viremia and shortening duration of viral shedding. They can provide an efficient protection against a lethal challenge with their respective parental HP-PRRSV isolates. We must clearly understand any one vaccine cannot completely prevent infection and establish sterilizing immunity, etc. PRRSV and PCV.

CONCLUSIONS

This study was the first detailed investigation into epidemiology of PCV3 and NADC30-like PRRSV under the field condition of immune PRRSV inactivated vaccine and PCV2 VLPs vaccine for 1 year in Jinzhong, Shanxi Province, China. During the 1-year surveillance period, the inactivated PRRSVVaccine and PCV2 VLPs vaccine could not provide virological protection to the piglets, and the piglets were still getting sick. In view of the continuous variation of PRRSVs and PCV virus genes, it is necessary to provide better immune adjuvants for obtaining better clinical immune protection.

ACKNOWLEDGMENTS

The authors would like to thank the staff at Shanxi Agricultural University (SAU; Taigu, China) who were directly involved in the production of their practical and professional assistance with vaccine management during the preparation of this review article.

AUTHORS'CONTRIBUTIONS

M.X. Zheng and L. Zhang edited the review. L.L. Zheng completed data collection and experimentation, prepared the main manuscript text and Figures 1-2 of the paper. H.X. Kang, F. Tan and C. Wang prepared Table 1-4. All authors reviewed and considered the manuscript.

FUNDING

College of Veterinary Medicine Scientific research innovation project, Shanxi Agricultural University(DY-Q002); 2. Graduate education innovation project of Shanxi Province in 2021,China(2021Y316); 3. Financial reward for doctoral graduates and researchers from Shanxi Province to work in Shanxi, China(SXBYKY2021041); 4. Shanxi Province Basic Research Program, China(20210302124495).

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