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Molecular cloning, functional characterization, tissue expression and polymorphism analysis of buffalo *PRDX6* gene

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Abstract: PRDX6 is a bifunctional protein involved in antioxidant regulation and phospholipid metabolism. Previous studies have shown that PRDX6 is involved in some biological pathways and networks related to lactation. The aim of this study was to explore the characteristics, function, tissue expression and variation of buffalo *PRDX6* gene. We cloned and characterized the complete coding sequence (CDS) of buffalo *PRDX6*. The CDS of *PRDX6* for swamp and river buffalo is the same, which consists of 675 nucleotides and encodes a protein of 224 amino acids. Buffalo PRDX6 contains one PRX_1cys functional domain (AA 7–222), which is probably related to the regulation of oxidative stress. Multi-tissue differential expression analysis showed that buffalo *PRDX6* was highly expressed in the muscle, brain, lung and small intestine during non-lactation and lactation, and there were significant differences in expression in all the tissues except the small intestine between the two periods. It is worth noting that the mRNA abundance of buffalo *PRDX6* in non-lactating mammary gland is higher than that in lactating mammary gland. Among the two single nucleotide polymorphisms (SNPs) identified in the CDS in this study, c.261C>T is shared by the two types of buffalo with different allelic frequencies, and c.426T>G is found only in river buffalo. The c.426T>G is non-synonymous, resulting in the amino acid substitution p.Asn142Lys. Only one nucleotide differential site is identified in *PRDX6* gene between buffalo and other species of *Bovidae*. Phylogenetic analysis indicated that buffalo PRDX6 has a closer genetic relationship with that of the species in *Bovidae*. These results indicate that PRDX6 probably plays a crucial role in the mammary gland of buffalo. This study provides the foundation for further functional studies of PRDX6 in buffalo.

Keywords: water buffalo; peroxiredoxin 6 gene; bioinformatic analysis; SNPs; population genetic structure

The peroxiredoxins (PRDXs), a newly-defined family of antioxidants, can prevent oxidative injury in cells. Based on the mechanism of peroxidase activity, PRDXs are further classified as 1-Cys and 2-Cys peroxiredoxins (Manevich and Fisher 2005). PRDX6 is the sole mammalian 1-Cys peroxiredoxin and exerts a significant role in maintaining cell homeostasis (Wu et al. 2015). As a bifunctional protein with both glutathione peroxidase and phospholipase A_2 activities, PRDX6 has the ability of antioxidant defence and phospholipid synthesis (Fisher 2011). It can inhibit oxidation by reducing membrane phospholipid peroxidation, and maintain the phospholipid homeostasis

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by generating lysophospholipid substrate for the remodelling pathway of phospholipid synthesis. In mice, the enzyme PRDX6 prevents oxidative injury in the mammary gland (Wang et al. 2003). In addition to oxidation-reduction, PRDX6 is also related to intracellular signal transduction (Perkins et al. 2015). In the PRDX superfamily, only PRDX6 can be modulated by cytokines (Paula et al. 2013). PRDX6 can affect the interaction between pro- and anti-inflammatory cytokines to determine the antioxidant capacity of cells. Thus, PRDX6 is implicated in development and progression of some diseases involved in oxidative stress (Wu et al. 2015).

The complete coding sequence (CDS) of bovine *PRDX6* gene is 675 bp in length, encoding a peptide consisting of 224 amino acid residues (Leyens et al. 2003). Bovine PRDX6 is distributed in cytosol, lysosome, nucleus and extracellular matrix (Leyens et al. 2003). The CDS of sheep *PRDX6* gene is the same in length as bovine *PRDX6* (Liu et al. 2015). Previous study has shown that the expression levels of mRNA and protein of PRDX6 in buffalo during lactation and non-lactation are significantly different, suggesting that the PRDX6 plays an important role in buffalo lactation (Jena et al. 2015).

The domesticated buffalo is grouped into river buffalo with karyotype 2n = 50 and swamp buffalo with 2n = 48, where the swamp buffalo is used as draft power while the river buffalo is mainly reared for milk production (Luo et al. 2020). Buffalo milk contains higher protein and fat relative to cow milk (D'Ambrosio et al. 2008), which makes buffalo milk have good processing characteristics. At present, about 15% of the milk produced in the world is sourced from buffalo (Anand et al. 2012). So far, there has been only some preliminary information about *PRDX6* gene in water buffalo (Jena et al. 2015). The primary purpose of this study was to investigate the molecular characteristics, function, tissue expression and population variation of *PRDX6* gene in water buffalo.

MATERIAL AND METHODS

Animals and sample collection

The samples used for the purpose of gene isolation and tissue expression analysis were collected from 13 adult dairy buffalo (about four-year-old), including five lactating Binglangjiang buffalo (river type) and three lactating Dehong buffalo (swamp type, about 60 days postpartum), and five non-lactating Binglangjiang buffalo (about 60 days before parturition). Binglangjiang buffalo and Dehong buffalo are famous indigenous buffalo breeds in western Yunnan. All buffalo had the same feeding and management conditions and were free to drink clean water. After the buffalo were slaughtered, the tissue samples were immediately excised from the mammary gland, muscle, small intestine, lung, liver, kidney, brain, heart, spleen and rumen, and stored in liquid nitrogen and transported back to the laboratory for total RNA extraction.

In addition, a total of 161 blood samples was collected from adult healthy buffalo for single nucleotide polymorphism (SNP) detection, among which 84 were from Binglangjiang buffalo and 77 from Dehong buffalo.

All the experimental procedures were approved by the Guide for Animal Care and Use of Experimental Animals of the Yunnan Provincial Experimental Animal Management Committee under Contract 2007-0069.

Total RNA extraction and gene isolation

Total RNA was extracted using TRIzol reagent (Invitrogen, Waltham, MA, USA) following the manufacturer's protocols. The concentration and purity of total RNA were determined by a NanoDrop 2000UV-Vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The cDNA was synthesized using a First Strand cDNA Synthesis Kit (TaKaRa, Dalian, P.R. China). The primers for amplifying *PRDX6* CDS were designed using Primer Premier 5.0 [Table S1 in electronic supplementary material (ESM); for the supplementary material see the electronic version] (Lalitha 2000) with the predicted mRNA sequence of buffalo *PRDX6* gene (No. XM_006063640). The mixed cDNA from each tissue was used as the template for PCR, and the reaction system and protocol were performed based on the manufacturers' instructions of 2× PCR Master Mix (CWBIO, Beijing, P.R. China). The purified PCR products were ligated into pMD-18T vector (TaKaRa, Dalian, P.R. China) and sequenced bidirectionally.

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Molecular characteristics analysis

The obtained sequence of buffalo *PRDX6* was determined using Editseq (DNASTAR, Inc., Madison, WI, USA). The gene was identified by homologous search in the BLAST program (http://www.ncbi.nlm.nih.gov/Blast.cgi). Physicochemical characteristics, hydropathy, subcellular localization, transmenbrane region and signal peptide were predicted using the ProtParam tool (https://web.expasy.org/protparam/), ProtScale (http://web.expasy.org/protscale/), ProtComp v9.0 (http://linux1.softberry.com/berry.phtml), TMHMM v2.0 (http://www.cbs.dtu.dk/services/TMHMM/) and SignalP v4.1 Server (http://www.cbs.dtu.dk/services/SignalP/), respectively. The conserved domain was analysed using the Conserved Domain Architecture Retrieval Tool of BLAST. The inferred secondary and tertiary structures of amino acid sequences were determined using SOPMA (http://npsa-pbil.ibcp.fr/) and SWISS-MODEL (http://swissmodel.expasy.org/). Biological process and molecular function analysis were performed using InterProScan (http://www.ebi.ac.uk/interpro/search/sequence-search). Furthermore, the WAG phylogenetic tree was established by the Mega 6 software based on the amino acid sequences of PRDX6 protein (Tamura et al. 2013).

Quantitative PCR (qPCR) and tissue differential expression

The gene expression of *PRDX6* in 10 tissues in lactation and non-lactation was conducted in accordance with the manufacturer's protocols of SYBR Green (TaKaRa, Dalian, P.R. China) on a CFX Connect Real-Time System (Bio-Rad Laboratories, Inc., Hercules, CA, USA). A pair of specific RT-qPCR primers was synthesized for the *PRDX6* gene according to the obtained CDS sequence of buffalo *PRDX6* in this study. After screening and evaluating the stability of gene expression, the geometric mean of the Ct values of the beta-actin (*ACTB*; accession No. NM_001290932), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*; accession No. XM_006065800) and ribosomal protein S23 (*RPS23*; accession No. XM_006059350) was used to normalize the targeted mRNA profiles (Table S1 in ESM). The qPCR raw data were analyzed by the $2^{-\Delta\Delta Ct}$ method. A statistical comparison of the means in multiple tissues between the two groups was determined via Student's *t*-test and the significance level was set to *P*-values lower than 0.05.

DNA extraction and polymorphism identification

Genomic DNA from the blood samples was extracted using TIANamp Genomic DNA Kit (TIANGEN Biotech Co., Ltd., Beijing, P.R. China) following the manufacturer's instruction. All the primers were designed (Table S1 in ESM) based on the sequence of buffalo *PRDX6* (accession No. NC_037549) and the PCR procedures were performed based on the manufacturer's instruction of 2× PCR Master Mix (CWBIO, Beijing, P.R. China). The PCR products were sequenced with the above PCR primers.

The position and number of SNPs were confirmed and exported with Seqman (DNASTAR, Inc., Madison, WI, USA) and Mega 6. The allelic and genotypic frequencies and Hardy-Weinberg equilibrium test were calculated by PopGen32 software (Yeh and Boyle 1997). The effect of amino acid substitutions on the protein function was evaluated using PROVEAN (http://provean.jcvi.org/index.php). The haplotypes of observed SNPs were predicted by PHASE software, and the number of iterations is ≥ 100 (Stephens et al. 2001).

RESULTS

Cloning and identification of buffalo *PRDX6*

A PCR product of 780 bp was obtained (Figure S1 in ESM). It was predicted that the obtained sequence contains a CDS of 675 bp. The CDS shares 99.11%, 98.81%, 98.96%, 99.41%, 99.41% and 98.96% similarity with cattle (NM_174643), zebu (XM_019976283), yak (XM_005890839), bison (XM_010850063), sheep (NM_001280704) and goat (XM_018060608), respectively. Therefore, the obtained CDS was determined to be the buffalo *PRDX6* gene (MH085036), which is identical with the predicted sequence of Mediterranean water buffalo XM_006063640.2 except for the two nucleotide differences at positions 261 and 462. Sequence alignment analysis showed that the nucleotide sequence of the *PRDX6* of river buffalo is the same as that of swamp buffalo. Buffalo *PRDX6*

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encodes a predicted polypeptide of 224 amino acid residues (AAs).

Physicochemical properties and protein structures of PRDX6

In order to better understand the function of buffalo PRDX6, we compared the physicochemical properties of PRDX6 between buffalo and cattle (accession no. NM_174643; Table 1). The results showed that buffalo PRDX6 had similar physicochemical properties to cattle PRDX6. The molecular weight was calculated as 25.05 kDa and the isoelectric point was predicted to be 5.73. Its grand average of hydropathicity is –0.308, suggesting that buffalo PRDX6 is a hydrophilic protein. Buffalo PRDX6 contains one conserved PRX_1cys (AA 7–222) functional domain without signal peptide or transmembrane region. The prediction of secondary structure indicated that the deduced buffalo PRDX6 contained 23.66% α-helix, 23.21% extended strand, 9.82% β turn and 43.30% random coils (Table S2 in ESM). Furthermore, tertiary structure analysis showed that the sequence consistency between buffalo PRDX6 and human PRDX6 (5b6n.3.B) is 94.64% and the coverage is 100% (Figure S2 in ESM).

Subcellular localization, biological process and molecular function

The potential protein subcellular localization analysis by ProtComp v9.0 showed that buffalo PRDX6 is probably located in nuclear (46.6%), mitochondrial (34.4%) and cytoplasmic compartments (18.7%). By the program InterProScan online, buffalo PRDX6 was predicted to involve in the biological process of oxidation-reduction (GO: 0055114). Its molecular functions are antioxidant activity (GO: 0016209), oxidoreductase activity (GO: 0016491) and peroxiredoxin activity (GO: 0051920).

Phylogenetic relationship

In order to explore the genetic relationships of PRDX6 protein among various species, a phylogenetic tree was reconstructed on the basis of amino acid sequences of 10 representative mammal animals. The result showed that the sequence consistency of PRDX6 protein among buffalo and the species of *Bovidae* is more than 99.1% (Table 2). The phylogenetic analysis displayed that buffalo and the other species of *Bovidae* clustered in one clade, indicating that buffalo PRDX6 has a closer genetic relationship with that of the species in *Bovidae* (Figure 1).

Tissue differential expression assays

To check the tissue differential expression of the *PRDX6* gene, we performed the qPCR analysis on multiple tissues of lactating and non-lactating river buffalo (Figure 2). As a result, buffalo *PRDX6* mRNA was distributed in all the examined tissues in these two periods. A relatively high level

Table 1. Physicochemical properties of PRDX6 for buffalo and cattle

Basic physicochemical properties	Buffalo	Cattle
Formula	$C_{1136}H_{1792}N_{296}O_{329}S_6$	$C_{1138}H_{1798}N_{296}O_{328}S_6$
Number of amino acids	224	224
Molecular weight (kDa)	25.05	25.07
Isoelectric point (pI)	5.73	6.00
Strongly acidic amino acids (D, E)	32	32
Strongly basic amino acids (K, R)	29	30
Polar amino acids (N, C, Q, S, T, Y)	41	40
Hydrophobic amino acids (A, I, L, F, W, V)	80	80
Instability index (II)	43.19	42.81
Grand average of hydropathicity (GRAVY)	–0.308	–0.310
Aliphatic index	88.84	88.84

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Table 2. Percent identity/divergence among the amino acid sequences of 10 representative mammal animals

Divergence	Percent identity											
	1	2	3	4	5	6	7	8	9	10		
1	–	99.1	99.6	99.6	99.6	99.1	96.9	92.0	90.2	94.6	1	Buffalo
2	0.9	–	99.6	99.6	99.6	99.1	96.9	92.0	90.2	94.6	2	Cattle
3	0.4	0.4	–	100.0	100.0	99.6	97.3	92.4	90.6	95.1	3	Yak
4	0.4	0.4	0.0	–	100.0	99.6	97.3	92.4	90.6	95.1	4	Bison
5	0.4	0.4	0.0	0.0	–	99.6	97.3	92.4	90.6	95.1	5	Sheep
6	0.9	0.9	0.4	0.4	0.4	–	96.9	92.0	90.2	95.1	6	Goat
7	3.2	3.2	2.7	2.7	2.7	3.2	–	91.1	89.7	93.3	7	Pig
8	8.5	8.5	8.0	8.0	8.0	8.5	9.5	–	93.3	91.5	8	Rat
9	10.6	10.6	10.0	10.0	10.0	10.6	11.1	7.0	–	89.7	9	Mouse
10	5.6	5.6	5.1	5.1	5.1	5.6	7.0	9.0	11.1	–	10	Human

The data of upper triangular represents the percent identity and the data of lower triangular represents the sequence divergence

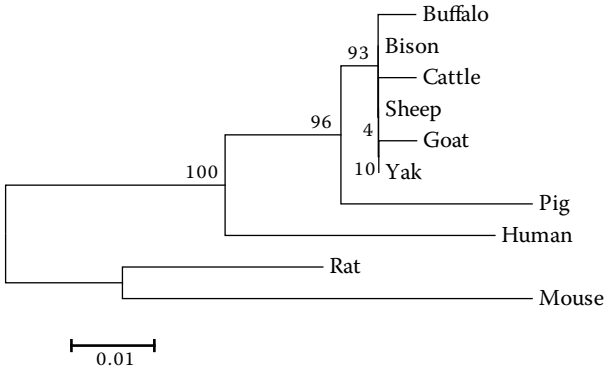

Figure 1. Phylogenetic tree based on the amino acid sequences of 10 representative mammal animals constructed by using the maximum likelihood method (WAG) The numbers adjacent to nodes represent bootstrap values for 10 000 replications

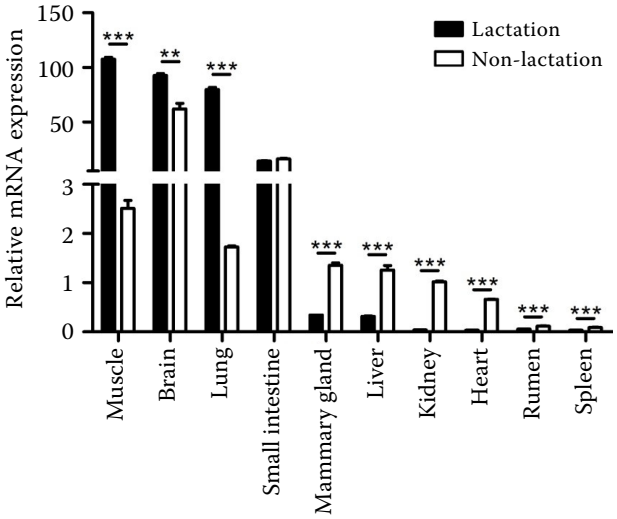

Figure 2. Relative mRNA expression of *PRDX6* in 10 tissues of lactating and non-lactating buffalo
Values are displayed with means ± SEM
$^{**}P < 0.01$; $^{***}P < 0.001$

of expression was observed in the muscle, brain, lung and small intestine. The abundance of *PRDX6* gene in the mammary gland, liver, kidney, heart, rumen and spleen in non-lactation was significantly higher than that in lactation ($P < 0.05$), while its abundance in the muscle, brain and lung was on the contrary (Figure 2). There was no significant difference ($P > 0.05$) in the mRNA expression of *PRDX6* in the small intestine between lactation and non-lactation.

Population variation analysis

Two nucleotide substitutions were identified in the CDS of buffalo *PRDX6* gene, referred to as MH085036.1: c.261C>T and MH085036.1: c.426T>G. The genotype and allele frequencies are provided in Table 3. The c.426T>G was found only in river buffalo (all the individuals for the swamp buffalo were homozygous TT), c.261C>T was shared by river and swamp buffalo. It should be noted that the high frequency alleles of c.261C>T in river and swamp buffalo are different. The test of Hardy-Weinberg equilibrium displayed that all SNPs are in equilibrium ($P > 0.05$).

By comparing the data in this work with buffalo *PRDX6* sequences downloaded from NCBI, the number of SNPs in buffalo *PRDX6* has not changed. It is worth noting that we found an A base at c.426 on the GenBank sequence XM_006063640.2. Among

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Table 3. Genotypic and allele frequencies for the single nucleotide polymorphisms (SNPs) identified in two types of buffalo

Population	SNP	Genotype	Frequency	Allele	Frequency	*P*-value[1]
River buffalo	c.261C>T	CC	0.154	C	0.307 7	0.244 3
		CT	0.308	T	0.692 3	
		TT	0.538			
	c.426T>G	TT	0.461	T	0.653 8	0.488 2
		TG	0.385	G	0.346 2	
		GG	0.154			
Swamp buffalo	c.261C>T	CC	0.545	C	0.727 3	0.428 7
		CT	0.364	T	0.272 7	
		TT	0.091			
	c.426T>G	TT	1.000	T	1.000 0	–

[1]*P*-value of Hardy-Weinberg equilibrium test

the nucleotide substitutions, c.261C>T is synonymous, while c.426T>G is non-synonymous, resulting in the amino acid substitution p.Asn142Lys (Figure 3 and 4). In silico prediction indicates that the substitution of p.Asn142Lys has no effect on the function of buffalo PRDX6.

Sequence differences in *PRDX6* haplotype

Four haplotypes (B1–B4) were constructed with the SNPs of the *PRDX6* gene in two types of buffalo (Figure 3), three of which (B1–B3) (accession numbers MH085036–MH085038; Table 4) were obtained from this work, and the other one was obtained from published data (accession number NC_037549). Among them, haplotypes B1–B2 were shared by two types of buffalo, while the oth-

```
                1111
                30247
                53227
B1              IIKNT
B2              .....
B3              ...K.
B4              ...K.
cattle hap1     ...KS
cattle hap2     ...K.
cattle hap3     ...K.
yak hap1        ...K.
bison hap1      ...K.
sheep hap1      ...K.
sheep hap2      MV.K.
goat hap1       ..RK.
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Figure 4. Differences in protein sequences corresponding to the haplotypes of PRDX6 in the species of Bovidae
B1–B4 are the haplotypes defined in buffalo. Number denotes the position of the coding region. Dots (.) represent identity with B1

```
                111 111 111 111 111 222 222 222 333 333 333 333 444 555 555 666
                000 000 111 222 999 566 666 888 000 666 666 777 222 233 333 333
            456 012 345 567 789 678 901 567 345 789 456 789 012 456 901 234 456
B1          CCC GGC ATT CAT TTT GTT AAC TAC ACA ATT AAA GAC GAA AAT ACC CCA CCA
B2          ... ... ... ... ... ... ..T ... ... ... ... ... ... ... ... ... ...
B3          ... ... ... ... ... ... ..T ... ... ... ... ... ... ..G ... ... ...
B4          ... ... ... ... ... ... ..T ... ... ... ... ... ... ..A ... ... ...
cattle hap1 ... ... ... ... ..C ..C ..T ... ... ... ... ... ... ..A T.. ..G ...
cattle hap2 ... ... ... ... ..C ..C ..T ... ... ... ... ..T ... ..A ... ..G ...
cattle hap3 ..T ... ... ... ..C ..C ..T ... ... ... ... ..T ... ..A ... ..G ...
yak hap1    ... ..T ... ... ... ..C ..T ..T ... ... ... ..T ... ..A ... ..G ...
bison hap1  ... ... ... ... ... ... ..T ... ... ... ... ..T ... ..A ... ..G ...
sheep hap1  ... ... ... ..C ... ... ... ... ... ... ... ... ... ..A ... ..G ..G
sheep hap2  ... ... ..G ..C ... ... ... ... ... G.. ... ... ... ..A ... ..G ..G
goat hap1   ... ... ... ..C ... ... ... ... ..G ... .G. ... ..G ..A ... ..G ..G
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Figure 3. Sequence differences in the haplotype sequences between buffalo and the other species of Bovidae
B1–B4 are the haplotypes defined in buffalo. Number denotes the position of the coding region. Dots (.) represent identity with B1

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Table 4. Haplotype frequencies of buffalo *PRDX6* gene

Haplotype	Alleles	Actual frequency	Expected frequency
B1	CT	0.500	0.497
B2	TT	0.313	0.315
B3	TG	0.187	0.185

ers were observed only in river buffalo. Multiple sequence alignments were performed to investigate the sequence differences in *PRDX6* gene between buffalo and other species in *Bovidae*. The accession numbers of the published representative haplotypes are EH146620, NM_174643, AF080228, XM_005890839, XM_010850063, NM_001280704, EE835548 and NC_030823. There is only one nucleotide difference between buffalo and other species of *Bovidae*, which is located at c.534 of the CDS, and this nucleotide difference does not lead to any amino acid difference (Figure 3 and 4).

DISCUSSION

In the present study, the *PRDX6* gene was identified from river and swamp buffalo. The CDS of this gene for both river and swamp buffalo has 675 nucleotides encoding a polypeptide of 224 amino acids, which has similar molecular characteristics to those of cattle PRDX6. Buffalo PRDX6 belongs to an unstable protein and is predicted to be located not only in the nucleus, but also in the mitochondria and cytoplasm, suggesting that it is likely to play a role in the nuclear, cytoplasmic and mitochondrial compartments, which is consistent with the results of bovine PRDX6 (Leyens et al. 2003). Previous studies have shown that PRDX6 is a bifunctional protein with the activity of glutathione peroxidase and phospholipase A_2, which are related to various biological processes, including an oxidation-reduction process (Fisher 2011). It has been confirmed that PRDX6 can reduce H_2O_2 and phospholipid hydroperoxides (Manevich and Fisher 2005), and exert an important function in the regulation of phospholipid turnover as well as in protection against oxidative damage. Therefore, PRDX6 is related to the process of lipid metabolism. The results of this work revealed that buffalo PRDX6 contains a PRX_1cys domain, and its molecular functions are mainly related to antioxidant activity, oxidoreductase activity and peroxiredoxin activity. The PRDX6 of buffalo and other species, especially cattle, have similar physicochemical characteristics and structures, and their sequence identity is high, suggesting that PRDX6 is functionally conservative and buffalo PRDX6 is likely to have similar functions to those of other animals in *Bovidae*. The above results indicate that buffalo PRDX6 may also be related to the regulation of oxidative stress and phospholipid synthesis.

In rat, PRDX6 protein was initially present in the lung tissue, but could not be detected in other tissues (Kim et al. 1998). But this seems to be due to the relatively low reactivity of the antibodies at that time. Recent studies have shown that rat PRDX6 protein is widespread in all major organs (Manevich and Fisher 2005). The detection of rat PRDX6 by Northern blot and Western blot showed that the expression was the highest in the lung, followed by the liver, brain, heart and kidney (Manevich and Fisher 2005). High expression of PRDX6 in the lungs could be related to its important function in the turnover of lung surfactant phospholipids and protective antioxidant function (Fisher 2018). It was found in sheep that PRDX6 protein is expressed in 10 tissues, among which the lung tissue has the highest expression and the muscle has the lowest expression (Liu et al. 2015). In this study, we found that the abundance of *PRDX6* mRNA of buffalo in the muscle, brain and lung of lactation and non-lactation period was high, indicating that this gene plays an important role in these tissues of buffalo in different physiological periods. However, the high expression level of *PRDX6* in buffalo muscle is inconsistent with the results of its low expression in the muscle shown in sheep. The high expression of *PRDX6* in muscle may be related to the antioxidant ability of muscle (Brinkmann et al. 2012). In some buffalo tissues, it was found that there was a significant difference in the mRNA abundance of *PRDX6* gene between lactation and non-lactation, and it was speculated that the gene may have different functions in different physiological states. It has been reported that *PRDX6* was involved in the lactation process (Jena et al. 2015). We detected the expression of *PRDX6* gene in the mammary tissue of lactating buffalo, and speculated that PRDX6 also exerts an important function in the buffalo lactation. It should be noted that the mRNA level of buffalo *PRDX6* here in the mammary gland of non-lactation was higher than that in the mammary gland of lactation, which is

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consistent with the previous reports in buffalo (Jena et al. 2015) and bovines (Dai et al. 2017), indicating that it might be important in mammary development before lactation.

Two potential SNPs (SNP417 and SNP423) were found in exon 4 of porcine *PRDX6*, and both of them were significantly associated with intramuscular fat, but they did not cause any amino acid changes (Liu et al. 2011). Nevertheless, there are few data about the polymorphisms of buffalo *PRDX6*. In the present study, two SNPs were found in the CDS of buffalo *PRDX6*, the SNP426 was nonsynonymous, but it does not affect the function of the PRDX6. The high conservatism of PRDX6 indicates that it probably exerts an important role in the physiological function of mammals. Whether the SNPs identified in buffalo have any effect on lactation should be further verified.

Sequence alignment indicates that the CDS length of buffalo *PRDX6* is the same as that of *Bovidae* species. There was only one nucleotide differential site in the CDSs of *PRDX6* gene between buffalo and other species of *Bovidae*, which further shows that *PRDX6* is functionally conserved. The phylogenetic analysis by using the sequences of PRDX6 displayed that buffalo had a closer genetic relationship with the other species of *Bovidae*, indicating that buffalo PRDX6 has minor functional divergence from the species of *Bovidae*. Furthermore, two nucleotide differential sites were found in the *PRDX6* gene between buffalo and the genus *Ovis*. These can be used as molecular markers to distinguish buffalo from the genus *Ovis*.

CONCLUSION

In this study, the *PRDX6* gene was isolated and identified from river and swamp buffalo. The molecular characteristics of PRDX6 of swamp and river buffalo are the same. In silico prediction indicates that PRDX6 protein is functionally conserved and has a high sequence consistency in the species of *Bovidae*. Buffalo PRDX6 can play a biological role in multiple tissues, such as lactation and development in the mammary gland. Two SNPs (SNP261 and SNP426) are found in the *PRDX6* CDS of buffalo. Among them, the c.426T>G is nonsynonymous, but it does not change the function of buffalo PRDX6. PRDX6 probably plays a crucial functional role in the mammary gland of buffalo. This study established the primary foundation for further exploring the function and variation of buffalo *PRDX6*.

Conflict of interest

The authors declare no conflict of interest.

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