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Original Article

Characterization and Phylogenetic Analysis of NDR Domain Protein From *Strongyloides ratti* Using Degenerate Primer PCR

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ABSTRACT

Introduction: The intestinal nematode *Strongyloides ratti* is an important and optimal model for the human pathogen, *Strongyloides stercoralis*. To design degenerate primers and amplify a putative NDR domain-containing gene fragment from *Strongyloides ratti* using touchdown reverse transcription polymerase chain reaction (RT-PCR).

Materials & Methods: A pair of degenerate primers was designed to amplify the target gene associated with nuclear Dbf2-related (NDR) domain-containing proteins, based on two conserved regions identified from related nematode protein sequences. A putative NDR domain-containing gene fragment of 249 bp from the *S. ratti* was amplified using Touchdown RT-PCR.

Results: The results showed that the amplified fragment between these two motifs from *S. ratti*, aligned with the C-terminus of the reference protein, shares 66.3% similarities. By searching the EST (expressed sequence tags) GenBank database, an overlapping 1082 bp cDNA fragment named SrNDR was compiled and found to contain a 972-nucleotide open reading frame encoding a protein of 324 amino acids, with an expected molecular mass of 23.4 kDa and calculatal pI of 7.64. The phylogram of SrNDR revealed that the gene variants of *S. ratti* SrNDR were grouped together with a strong bootstrap score of 87. Domain search analysis showed an e-value of 1.05e-100 for the conserved NDR1 domain within the a/b hydrolase superfamily protein (pfam03096), spanning amino acid residues 9 to 287. The core domain of SrNDR is folded into 12 alpha-helices surrounded by eight antiparallel b-strands. The three-dimensional structure model of SrNDR (residues 31-319) showed 100% identity to the human protein NDRG1 across 248 amino acids, with 77% sequence coverage.

Conclusion: By using this program, we found a prediction of predominantly a-helical (44.86%), Strand (1.08%) and random coil (54.05%) content for the coding sequence of SrNDR. Based on the alignment of this protein with homologous sequences from related nematodes, it may play a vital role in parasite survival within host cells.

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1. Introduction

The intestinal nematode *Strongyloides ratti*, a common parasite of rats, is a parasitic nematode with a unique life cycle characterized by obligate parasitic and facultative free-living generations [1, 2]. This parasite is important as a suitable model organism, as it is genetically very similar to the human pathogen, *Strongyloides stercoralis*.

The N-myc downstream regulated gene (*NDRG*) protein family consists of four members, *NDRG1-4* in humans, with 57-65% amino acid homology [3]. They belong to the a/b hydrolase superfamily (ABHD), which is well conserved through evolution. It has been shown that the excretory and secretory (ES) products of parasitic nematodes such as *Haemonchus contortus* [4], *Heligmosomoides polygyrus*[5], and *Mesocestoides corti* [6] are rich in ABHD proteins. Similar expressions of ABHD proteins and their homologs have also been demonstrated in free-living and parasitic organisms, such as *Caenorhabditis elegans* ABHD5 [7], *Cryptosporidium parvum* type II thioesterase (CpTEII) [8], and *Schistosoma japonicum* lysophospholipase [9]. Apart from the expression of ABHD proteins in a wide range of mammals, the expression of small amount of these proteins and their homologs in plants—such as *Arabidopsis thaliana* ABHD11 and ABHD5 [10, 11] —and in *Saccharomyces cerevisiae* ABHD5 homologs [12] has also been reported. Members of this family are also found in a wide variety of multicellular eukaryotes, including an NDR-1 type protein in *Helianthus annuus* (common sunflower), known as Sf21.

Although the exact function of NDR domain-containing proteins has not been clearly explained, recent evidence shows that mutations in these genes are associated with different neurological and physiological complications. It is predicted that this protein is a cytoplasmic and involved in energy metabolism, stress responses, hormone signaling, cell signaling, growth, and differentiation [13]. *NDRG1* is primarily localized in the cytoplasm, followed by its localization in the nucleus and mitochondrion at probabilities of 47.8%, 26.1% and 8.7%, respectively. *NDRG1* is translocated from the cytoplasm to the nucleus in response to DNA damage and hypoxia [14]. As reviewed by Fang et al., *NDRG1* is also involved in embryogenesis and development, cell growth and differentiation, lipid biosynthesis and myelination, stress responses, immunity, DNA repair, and cell adhesion, among other functions. As shown in the literature review [15, 16], *NDRG1* has been shown to be an effective inhibitor of metastasis signaling in a number of invasive cancers, such as prostate, pancreatic, breast, and colon cancer.

In this study, we aimed to perform a preliminary characterization of an NDR domain-containing protein from *S. ratti* to gain a better understanding of parasite-host interactions.

2. Materials and Methods

2.1. RNA isolation and cDNA synthesis

Total RNA from *S. ratti* larvae was prepared using RNX plus solution (CinnaGen, Iran) according to the manufacturer's instructions. The concentration of extracted RNA was measured by absorbance at 260 nm and either utilized directly or kept at -80 ˚C. Briefly, 12 µL (2 µg each) of total RNA was incubated with 0.5 µg of ModT (modified oligodT) primer (5′-GGGTCTAGAGCTCGAGTCACTTTTTTTTTTTTTTT-3′) at 70 ˚C for 10 min. The reaction was placed on ice before adding 1 µL RNasin (CinnaGen, Iran), 1 µL dNTP mixture (120 mM of each nucleotide), 2.5 µL of 5X enzyme buffer, and 1 µL (200 U) of Moloney Murine Leukemia Virus (M-MulV) reverse transcriptase (CinnaGen, Iran). The reaction was incubated at 42 ˚C for 1 h before inactivation at 70 ˚C.

2.2. Sequences obtained from GenBank database

Sequences associated with NDR domain-containing proteins were obtained from the GenBank database, following an extensive study of target genes in several nematode species. The assemblies from different species within the Strongyloides family of were retrieved to craete a database of proteins associated with *S. ratti*. The maximum accepted e-value in this study was 8e-07; sequence scores less than this value were rejected.

2.3. Reverse transcription polymerase chain reaction (RT-PCR) amplification using degenerate primers

Based on conserved sequences, two degenerate primers were designed. To generate a homologous DNA fragment for isolating the *S. ratti* NDR domain-containing protein, a fragment of the gene was amplified from cDNA using PCR with degenerate primers [17]. Primer sequences were: Sense primer sequence NDR-F (5′-GCGgaattcGGNGCNTGGGAYTA), related to the N-terminal region of the protein (GAWDY), and antisense primer sequence NDR-R (5′-GCGaagcttCCRCANCCYTTRCA), related

to the C-terminal region of the protein (CKGCG). (N, R, and Y represent A/G/C/T, A/G, and C/T, respectively). To facilitate directional cloning, EcoRI and BamHI were introduced at the 5′ends of the forward and reverse primers, respectively. Semi-nested RT-PCR reactions were performed on cDNA using touchdown PCR (TD-PCR) conditions. In order to facilitate Touchdown Semi-Nested RT-PCR, cDNA was produced using the modified oligo(dT) (Mod-T). This technique enabled the amplification of the correct transcripts in a two-round PCR protocol. For the first round of amplification, the ModT-R primer (5′-CCCAGATCTCGAGCTCAGTG) was designed. This complemented the 5'-end tail of the modified oligodT (ModT) primer. TD-PCR reactions were performed under the following conditions: 94 ˚C×3 min for one cycle; 94 ˚C × 30 s, 42 ˚C × 50 s, 72 ˚C × 1 min for 15 cycles, with a 1 ˚C increase per cycle; 94 ˚C × 30 s, 57 ˚C × 50 s, 72 ˚C× 40 s for 20 cycles. The first round of Touchdown PCR (TD-PCR) was performed using ModT-R and NDR-F primers with the original cDNA as template. The second round of PCR was performed using NDR-F and NDR-R primers with a one-tenth dilution of the first round PCR product as template. The PCR conditions for the second rounds were 30 cycles with denaturation at 94 ˚C for 30 seconds, annealing at 57 ˚C for 30 seconds, extension at 72 ˚C for 1 min, and a final extension at 72 ˚C for 5 min. The PCR reaction was performed using cDNA from *S. ratti* as the template with the following mix: 20 mM Tris-HCl, 50 mM KCl, 1.4 mM $MgCl_2$, 0.2 mM of each dNTPs, 0.5 U Taq polymerase, 0.4 µM of each primer, and 5 µL of cDNA. Analytical agarose gel containing 50 mg/mL of safe stain was prepared. PCR products were separated on 1% agarose gel by electrophoresis and visualized using a UV transilluminatore.

2.4. DNA sequence analysis

The amplified cDNA fragments were sequenced in both directions using the dideoxy termination method and run on an Applied Biosystems 373 DNA sequencer. The complete cDNA was determined using overlapping fragments. Primers were designed using the Primer3 program. The sequence was compared with database entries using the BLAST algorithm from the NCBI website [18]. To evaluate the evolutionary relationships between NDR domain-containing genes, target sequences from other species were retrieved from the NCBI GenBank. The putative signal peptides were analyzed using SignalP software [19]. Multiple sequence alignments were performed using the CLUSTAL_W program [20], and edited with BOXSHADE software [21]. The CDD-Search software from the NCBI site was used to identify conserved domains [22]. The molecular weight (MW) and theoretical isoelectric point (pI) of the deduced amino acid sequences were calculated using the export protein analysis program [23]. The secondary structure of the protein was predicted using the PSIPRED Protein Sequence Analysis Workbench [24]. The DAS-Transmembrane Prediction server [20] was used. The 3D structure prediction was performed using the Phyre2 program [26]. Phylogenetic analysis and genetic distance were evaluated using the neighbor-joining method with 1000 bootstrap replicates, implemented in MEGA11 software [27].

3. Results

3.1. Semi-nested RT-PCR amplification using degenerate primers

The NCBI GenBank protein database was searched using the keyword "NDR domain-containing protein nematodes". Twelve protein sequences including *Wuchereria bancrofti* (EJW87670.1), *Brugia malayi* (XP_001899587.2), *Litomosoides sigmodontis* (VDM91312.1), *Loa loa* (XP_020302625.1), *Acanthocheilonema viteae* (VBB26456.1), *Onchocerca flexuosa* (VDO39025.1), *Litomosoides sigmodontis* (VDM91312.1), *Ditylenchus destructor* (KAI1725449.1), *C. elegans* (NP_510634.1), *H. contortus* (CDJ84079.1), *Nippostrongylus brasiliensis* (WKY14995.1), *Oesophagostomum dentatum* (KHJ98162.1), and *Cylicocyclus nassatus* (CAJ0609661.1) were retrieved and used in the alignment. Based on multiple sequence of reference sequences, two highly conserved blocks of protein sequences —GAWDY (for the sense primer) and CKGCG (for the antisense primer) —were identified. Degenerate primers were designed from regions corresponding to amino acid positions approximately 111-115 for the sense primers and 223-227 for the antisense primers (Figure 1).

Those two blocks were translated using the International Union of Pure and Applied Chemistry (IUPAC) coding system. Some amino acids are encoded for by more codon triplets than others. The fold of degeneracy for each primer was estimated by multiplying the degeneracy values of individual amino acids according to the IUPAC coding system. The total fold of degeneracy for both primers was calculated to be 32. Starting with 0.5 g of larval sample, four µg of total RNA was extracted, and cDNA was synthesized. TD-PCR started with a low annealing temperature of 42 ˚C for 15 cycles, followed by 20 cycles at a high annealing temperature of 57 ˚C. The low annealing temperature permits short conserved primer regions to hybridize to their complementary strands. By increasing the annealing temperature, primer specificity is increased.

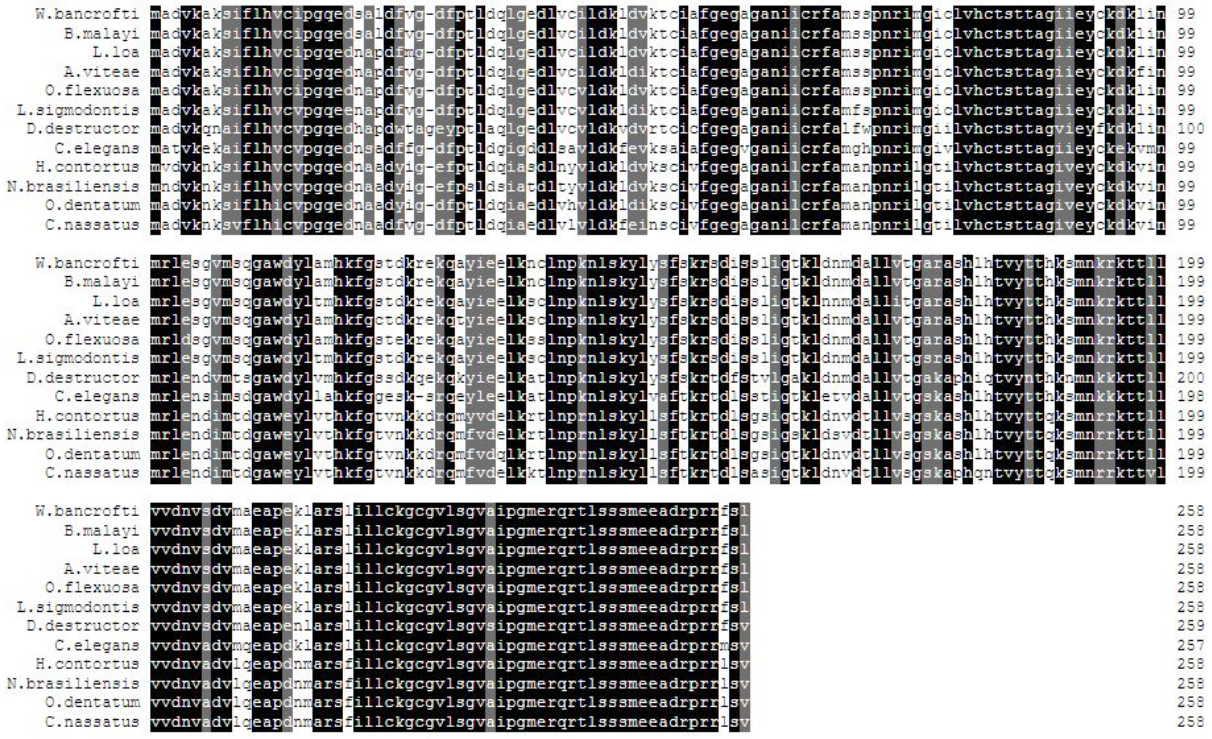


Figure 1. Alignments of nematode NDR domain-containing proteins related to *S. ratti* for designing degenerate primers

Note: Shading indicates identity (black) or conservative substitutions (grey). The conserved amino acids for designing degenerate primers are indicated by arrows.

3.2. Identification of NDR domain-containing gene

The degenerate primers amplified a putative NDR domain-containing gene fragment of 340 bp from the *S. ratti* cDNA template during the second round of PCR. The amplified partial NDR-containing gene from *S. ratti* was confirmed by sequence analysis. The amplified DNA contained an open reading frame encoding 116 amino acids, showing sequence identity to other NDR domain-containing proteins. This is in accordance with the expected amplicon size predicted by manual calculation. Sequence comparison was performed using the BLAST program on the NCBI GenBank database. In order to compare the nucleotide sequence of SrNDR domain-containing gene with sequences available in the GenBank database, the blastn program on the NCBI website was used with the "Highly similar sequences (megablast)" option. Comparisons of the amplified cDNA fragment sequence using the blastn program showed 99% similarity to two sequences from *S. ratti*: a 975 bp partial mRNA sequence (XM_024650741.1) and a 513 bp genomic assembly (LN609396.1). These two sequences are nearly identical. Searching the EST (expressed sequence tag) showed three overlapping sequences —BI323694.1 (496 bp), FC811605.1 (666 bp), and FC817168.1 (652 bp). An overlapping 1082 bp cDNA fragment, named SrNDR, was compiled and found to contain a 972-nucleotide open reading frame encoding a protein, with an expected molecular mass of 23.4 kDa and a calculated pI of 7.64 (Figure 2). Based on sequence alignments, the fragment includes the C-terminal region of the protein. Domain database searches showed an e-values of 1.05e-100 with the conserved NDR1 containing domain of the alpha/beta hydrolase superfamily protein (pfam03096), spanning amino acid residues 9 to 287 [22].

The assembled nucleotide sequence showed 84–99% identity with five NDR-containing partial mRNA sequences. The taxonomic analysis of SrNDR showed similarity to five sequences from the family Strongyloididae, including four belonging to the genus *Strongyloides*. It was revealed that *S. ratti*, with two sequence hits, showed the highest similarity within this family, while only one hit was associated with the *S. papillosus* species (Table 1). *Parastrongyloides trichosuri* showed the least similarity, with a single sequence hit.

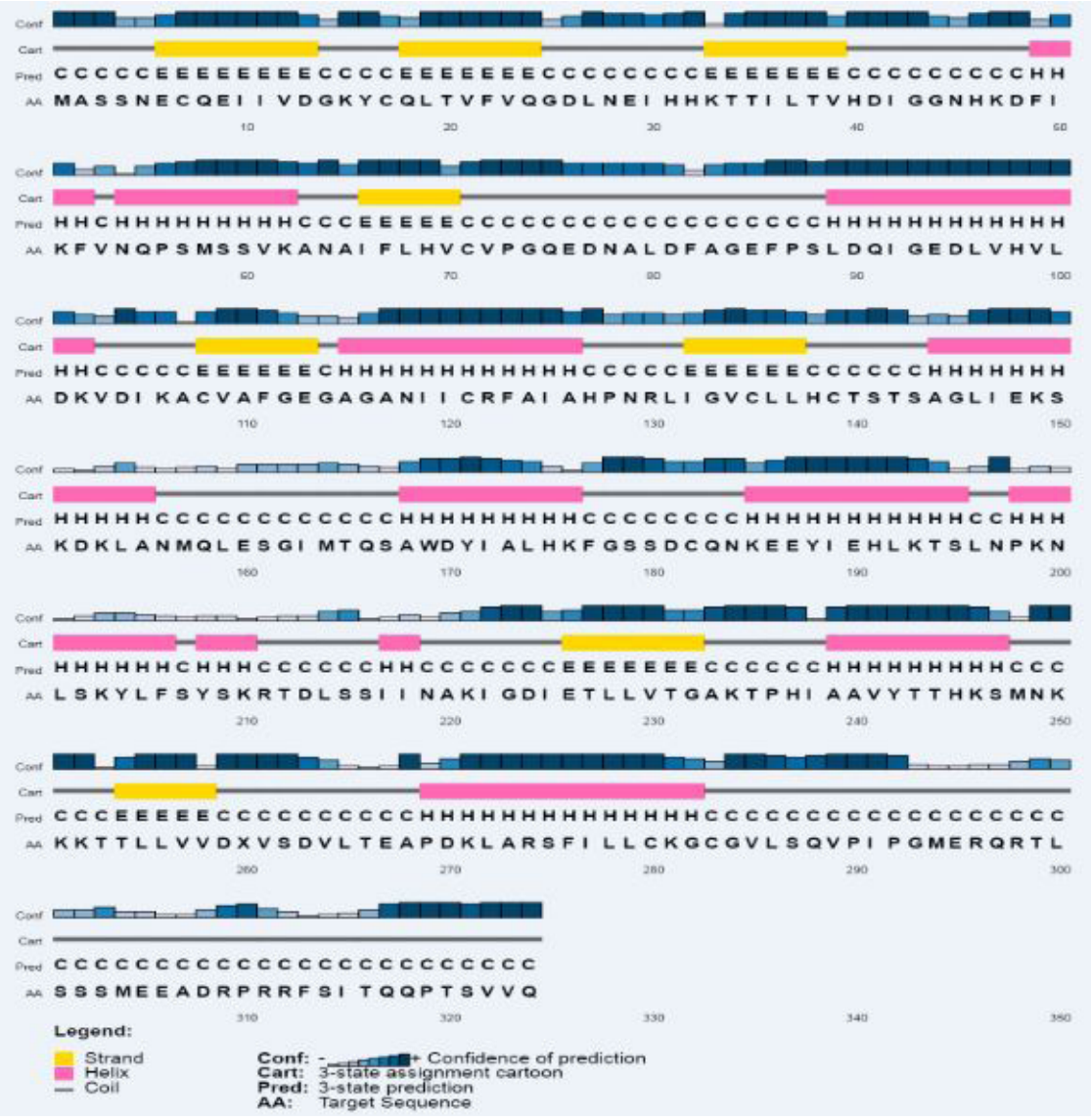


Figure 2. A schematic representation of SrNDR domain

3.3. Secondary structure and amino acid characterization

The PSIPRED protein sequence analysis method predicted the secondary structure of the SrNDR protein. Each residue was assigned propensity values for alpha helices, beta sheet and coils, using a sliding window of seven residues (Figure 3A). Using these parameters, the probability of each residue adopting a specific secondary structure was calculated, and the conformation with the highest confidence was predicted. Based on the secondary structure data and the computational three-dimensional structure model (Figure 3B), the protein was predicted to fold into 12 alpha-helices and eight antiparallel beta-strands. The three-dimensional structure folded core domain of SrNDR (residues 31-319) showed 100% sequence similarity with the human protein NDRG1 across 248 amino acids with, 77% coverage. The core of SrNDR is composed of an alpha/beta hydrolase fold, featuring a central eight-stranded b-sheet surrounded by alpha helices.

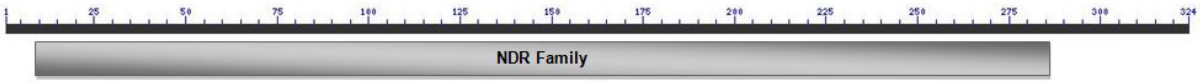
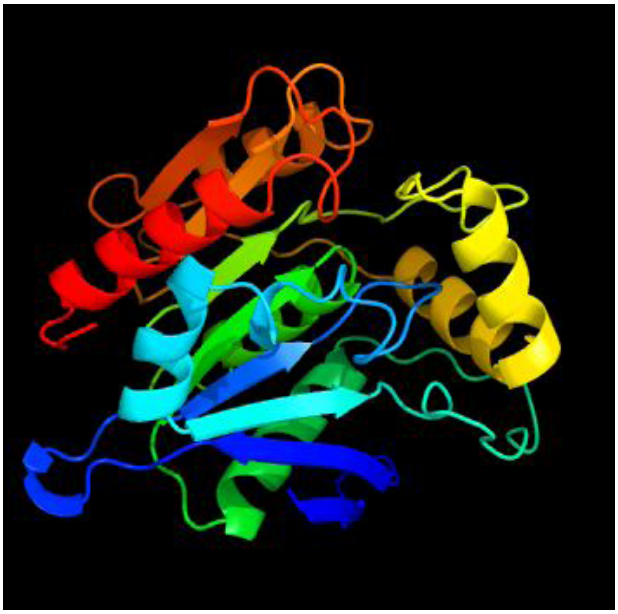


Figure 3. A schematic representation of the secondary structure (A) and three dimensional model (B) of SrNDR



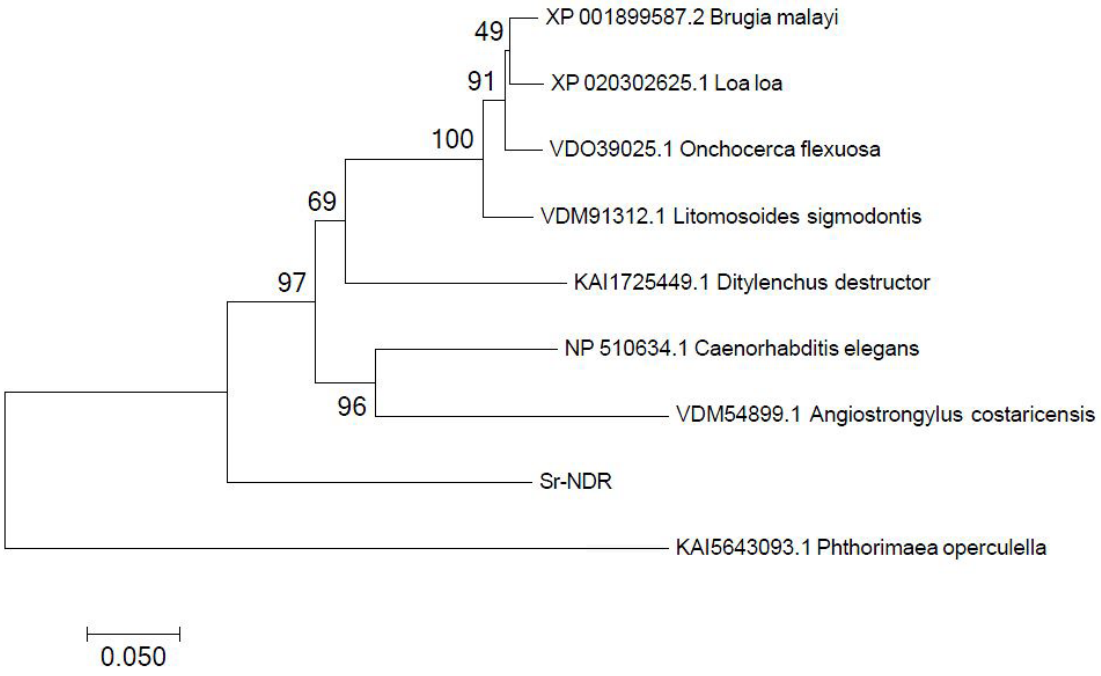


Figure 4. Phylogenetic analysis of SrNDR nucleotide sequences with homolog sequences using the Neighbor-joining method

Note: Bootstrap value is based on 1000 replicates. The numbers in front of the species are the accession numbers of the related gene variants in the GenBank. The numbers above the lines indicate the relationship between the groups.

Using this program, we predicted that the coding sequence of SrNDR is composed predominantly of alpha-helices (44.86%), with beta strands (1.08%) and random coils (54.05%) (Figure 3A). The three-dimensional (3D) structure of the SrNDR protein was modeled using 181 amino acids, 98% of the entire coding sequence, with 100% confidence based on the single highest-scoring template [26] (Figure 3B). DAS-transmembrane analysis of the SrNDR amino acid sequence identified three putative transmembrane domains, located between residues 65-69, 131-140, and 277-285. This analysis suggests that SrNDR may function as a transmembrane protein; however, its subcellular localization remains unknown.

3.4. Phylogenetic analysis

The phylogenetic tree of SrNDR nucleotide sequence was performed by the Neighbor-Joining method using MEGA11 software with all five homologous sequences that were observed in GenBank. Phylogenetic analysis

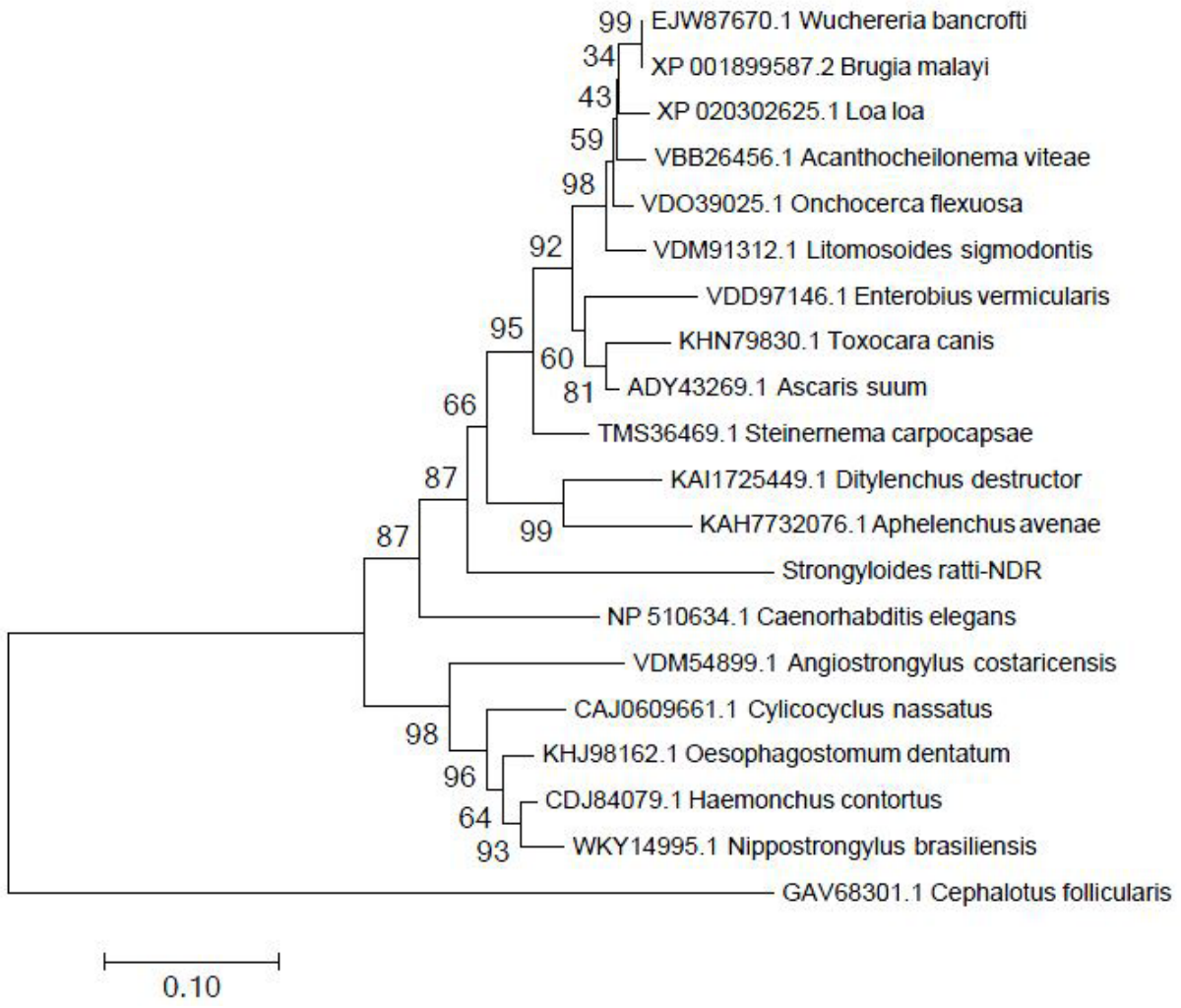


Figure 5. Phylogenetic tree constructed from the amino acid sequences of SrNDR with the related sequences using Neighbor-joining analysis

Note: Bootstrap numbers are based on 1000 replicates. The numbers in front of the species are the accession numbers of the related genes in the GenBank. The numbers above the lines indicate the relationship between the groups.

clearly showed an evolutionary relationship of SrNDR with the only two homologous sequences from *S. ratti* (one partial mRNA, and the other a genome assembly), revealing that this sequence was closely related to the Strongyloides genus but divergent from other parasitic nematode orthologs, such as *P. trichosuri* (Figure 4). Among the five nucleotide sequences given in Table 1, SrNDR and two sequences from *S. ratti* (XM 024650741.1 and LN609396.1) with high similarity are in the same cluster. However, the sequence from S. stercoralis (LL999077.1), with bootstrap value of 65, is separated from both of them. The other sequence, from *Strongyloides papillosus* (LM525602.1), was placed in a separate cluster. The *P. trichosuri* (LM523192.1) sequence is used as an outgroup.

In order to analyze SrNDR in the larger context of NDR domain-containing proteins, phylogenetic analysis was conducted based on the deduced amino acid sequences from *S. ratti* along with 18 sequences from the parasitic nematode family, as shown in Figure 5. The constructed phylogram showed two distinct clusters (A and B). In cluster A, the gene variant of *S. ratti* SrNDR is grouped together with mostly filarial and nematode parasites, with a bootstrap score of 87. Within this cluster, SrNDR formed a separate branch. As expected, The SrNDR sequence used in this study is perfectly arranged in cluster A, showing higher genetic similarity with different NDR domain-containing protein homologs. All intestinal nematode parasites belonging to cluster B are placed together with a strong bootstrap score of 98. *Cephalotus follicularis* (GAV68301.1) is used as an outgroup.

Table 1. Taxonomy report of SrNDR sequence based on blastn program

Organism	Blast Name	Score	# of Hits
Strongyloididae	Nematodes		5
Strongyloides	Nematodes		4
S.ratti BcDNA.GH02439 (SRAE_X000159500), partial mRNA	Nematodes	1786	2
S. stercoralis genome assembly S_ratti_ED321, scaffold srae_chrx_scaffold0000002	Nematodes	743	1
S. papillosus genome assembly S_papillosus_LIN, scaffold SPAL _ scaffold0000034	Nematodes	616	1
P. trichosuri genome assembly P_trichosuri_KNP, scaffold PTRK _ contig0000003	Nematodes	505	1

The genetic distance of SrNDR was calculated among NDR-domain containing nucleotide variant sequences using MEGA11 software. According to Table 2, among the five NDR-containing nucleotide sequences available in GenBank, SrNDR had the highest and lowest genetic distance with *S. papillosus* (LM525602.1) (10.6%) and the sequences from *S. ratti* (XM 024650741.1 and LN609396.1) (0.3%), respectively.

4. Discussion

Members of the *NDRG1* family all possess an NDR domain within esterases, lipases, proteases, peroxidases, dehalogenases, and epoxide hydrolases, which belong to the alpha/beta hydrolase superfamily (ABHD) proteins that are highly conserved and ubiquitously distributed throughout organisms [28]. It is suggested that this gene may act as a stress response or potentially as a transcription factor [29, 30]. However, the precise molecular and cellular function of these family members is still unknown.

Parasitic helminths have evolved sophisticated and highly complex mechanisms for cohabitation with hosts in different environments [31]. They can release ES proteins into the host environment to suppress the host immune response so as to ensure their survival [32]. It has been reported that one of the components of ES isolated from *H. contortus* was a member of the NDR domain-containing protein family that interact with host T cells [33]. Currently, to our knowledge, no structural characterization of this protein has been performed from *S. ratti*. Since NDR domain-containing proteins are relatively conserved with each other within the ABHD superfamily [28], we decided to isolate the counterpart of this protein from *S. ratti*.

Degenerate primers are commonly used for the isolation of unidentified gene sequences in related organisms. This allows us to discover unknown sequences of new members of gene families with unknown molecular functions. We designed degenerate primers from several known sequences associated with NDR domain-containing proteins in different nematode species related to target genes in *S. ratti*. The alignments of the selected sequences revealed two highly conserved motif regions of amino acids that enabled us to design degenerate primers. By synthesizing the degenerate primers, the conserved gene sequence fragments from the related organisms were isolated using degenerate primers-based polymerase chain reaction technique. At first, a primer pair standard PCR was used to isolate the gene frag-

Table 2. The genetic pairwise distances of SrNDR compared to the related nematode variant nucleotide sequences

Organisms	1	2	3	4	5	6
1. SR-NDR_*S. ratti*-1						
2- XM_024650741.1_*S. ratti*	0.003					
3- LN609396.1_*S. ratti*	0.003	0.000				
4- LL999077.1_*S. stercoralis*	0.071	0.068	0.068			
5- LM525602.1_*S. papillosus*	0.106	0.104	0.104	0.106		
6-LM523192.1_*P. trichosuri*	0.367	0.365	0.365	0.395	0.395	

ment, which was not successful. Since it was speculated that this lack of success might be due to the low level of expression of this gene, the amount of cDNA template was increased in each reaction. Again, no product was obtained. Therefore, we decided to perform a TD-PCR technique. In this technique, the temperature selected for the annealing step is initially set 5 ˚C lower than the calculated melting point of the primers. Annealing under conditions of low stringency allows the formation of more primer-template hybrids.

Multiple alignment and phylogenetic analysis of SrNDR based on nucleotide and amino acid sequences revealed that the gene encodes an NDR domain-containing protein. Based on the phylogenetic analysis with helminth homologs, SrNDR was predicted to belong to the ABHD subfamily. SrNDR possessed no signal peptide, suggesting that this reported gene is incomplete in the N-terminal part, or the protein, being an intracellular protein, may not be secreted from cells.

The function of NDR family proteins in the cell has not yet been well defined. However, it is believed that the proteins of this family of putative signaling molecules have an essential role in the host-parasite relationship. The role of NDR1 as a representative of a large group of ABHD family proteins, whose function is involved in the control of programmed cell death in *Arabidopsis*, has been reported [27]. By comparing the role of HcABHD protein in *H. contortus*, particularly its involvement in cell proliferation and apoptosis, SrNDR could also be one of the proteins that may play a vital role in the control of the cell cycle and the survival of key host cells.

5. Conclusion

By comparing the role of HcABHD protein in H. contortus, particularly its involvement in cell proliferation and apoptosis, SrNDR could also be one of the proteins that may play a vital role in the control of the cell cycle and the survival of key host cells.

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Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

Data availability

The data supporting the findings of this study are available from corresponding author upon reasonable request.

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Conflict of interest

The author declared no conflict of interest.

References

[1] Bethony J, Brooker S, Albonico M, Geiger SM, Loukas A, David Diemert D, et al. Soil-transmitted helminth infections: Ascariasis, trichuriasis, and hookworm. Lancet. 2006; 367(9521):1521-32. [DOI:10.1016/S0140-6736(06)68653-4] [PMID]

[2] Buonfrate D, Bisanzio D, Giorli G, Odermatt P, Fürst T, Greenaway C, et al. The global prevalence of strongyloides stercoralis infection. Pathogens. 2020; 9(6):468. [DOI:10.3390/pathogens9060468] [PMID]

[3] Okuda T, Kondoh H. Identification of new genes ndr2 and ndr3 which are related to Ndr1/RTP/Drg1 but show distinct tissue specificity and response to N-myc. Biochem Biophys Res Commun. 1999; 266(1):208-15. [DOI:10.1006/bbrc.1999.1780] [PMID]

[4] Wang T, Ma, G, Ang CS, Korhonen PK, Xu R, Nie S, et al. Somatic proteome of Haemonchus contortus. Int J Parasitol. 2019; 49(3-4):311-320. [DOI:10.1016/j.ijpara.2018.12.003] [PMID]

[5] Moreno Y, Gros PP, Tam M, Segura M, Valanparambil R, Geary TG, et al. Proteomic analysis of excretory-secretory products of Heligmosomoides polygyrus assessed with next-generation sequencing transcriptomic information. PLoS Negl Trop Dis. 2011; 5(10):e1370. [DOI:10.1371/journal.pntd.0001370] [PMID]

[6] Vendelova E, Camargo de Lima J, Lorenzatto KR, Monteiro KM, Mueller T, Veepaschit J, et al. Proteomic analysis of excretory-secretory products of Mesocestoides corti metacestodes reveals potential suppressors of dendritic cell functions. PLoS Negl Trop Dis. 2016; 10(10):e0005061. [DOI:10.1371/journal.pntd.0005061] [PMID]

[7] Xie M, Roy R. The causative gene in chanarian dorfman syndrome regulates lipid droplet homeostasis in C. elegans. PLoS Genet. 2015; 11(6):e1005284. [DOI:10.1371/journal.pgen.1005284] [PMID]

[8] Guo F, Zhang H, Eltahan R, Zhu G. Molecular and biochemical characterization of a type II thioesterase from the zoonotic protozoan parasite Cryptosporidium parvum. Front Cell Infect Microbiol. 2019; 9:199. [DOI:10.3389/fcimb.2019.00199] [PMID]

[9] Fan J, Yang W, Brindley PJ. Lysophospholipase from the human blood fluke, Schistosoma japonicum. Int J Infect Dis. 2008; 12(2):143-51. [DOI:10.1016/j.ijid.2007.05.005] [PMID]

[10] Vijayakumar A, Vijayaraj P, Vijayakumar, AK, Rajasekharan R. The Arabidopsis ABHD11 mutant accumulates polar lipids in leaves as a consequence of absent acylhydrolase activity. Plant Physiology. 2016; 170(1):180-93. [DOI:10.1104/pp.15.01615] [PMID]

[11] Park S, Gidda SK, James CN, Horn PJ, Khuu N, Seay, DC, et al. The a/b hydrolase CGI-58 and peroxisomal transport protein PXA1 coregulate lipid homeostasis and signaling in Arabidopsis. Plant Cell. 2013; 25(5):1726-39. [DOI:10.1105/tpc.113.111898] [PMID]

[12] Ghosh AK, Ramakrishnan G, Chandramohan C, Rajasekharan R. CGI-58, the causative gene for Chanarin-Dorfman syndrome, mediates acylation of lysophosphatidic acid. J Biol Chem. 2008; 283(36):24525-33. [DOI:10.1074/jbc.M801783200] [PMID]

[13] Qu X, Zhai Y, Wei H, Zhang C, Xing G, Yu Y et al. Characterization and expression of three novel differentiation-related genes belong to the human NDRG gene family. Mol Cell Biochem. 2002; 229(1-2):35-44. [DOI:10.1023/A:1017934810825] [PMID]

[14] Bae DH, Jansson PC, Huang ML, Kovacevic Z, Kalinowski D, Lee, CS, et al. The role of NDRG1 in the pathology and potential treatment of human cancers. J Clin Pathol. 2013; 66(11):911-7. [DOI:10.1136/jclinpath-2013-201692] [PMID]

[15] Kovacevic Z, Richardson DR. "The metastasis suppressor, Ndrg-1: A new ally in the fight against cancer". Carcinogenesis. 2006; 27(12):2355-66. [DOI:10.1093/carcin/bgl146] [PMID]

[16] Sahni S, Park KC, Kovacevic Z, Richardson DR. "Two mechanisms involving the autophagic and proteasomal pathways process the metastasis suppressor protein, N-myc downstream regulated gene 1". Biochim Biophys Acta Mol Basis Dis. 2019; 1865(6):1361-78. [DOI:10.1016/j.bbadis.2019.02.008] [PMID]

[17] Jolodar A. Designing of Degenerate Primers-Based Polymerase Chain Reaction (PCR) for Amplification of WD40 Repeat-Containing Proteins Using Local Allignment Search Method. J Sci. 2019; 30(2):105-11. [DOI:10.22059/jsciences.2019.271719.1007349]

[18] Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990; 215(3):403-10. [DOI:10.1016/S0022-2836(05)80360-2] [PMID]

[19] Nielsen H, Engelbrecht J, Brunak S, von Heijne G. Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. Protein Eng. 1997; 10(1):1-6. [DOI:10.1093/protein/10.1.1] [P MID]

[20] Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 1994; 22(22):4673-80. [DOI:10.1093/nar/22.22.4673] [PMID]

[21] Stothard P. The sequence manipulation suite: JavaScript programs for analyzing and formatting protein and DNA sequences. Biotechniques. 2000 Jun;28(6):1102, 1104. [DOI:10.2144/00286ir01]. PMID: 10868275.

[22] Marchler-Bauer A, Bo Y, Han L, He J, Lanczycki CJ, Lu S, et al. CDD/SPARCLE: Functional classification of proteins via subfamily domain architectures. Nucleic Acids Res. 2017; 45(D1):D200-3. [DOI:10.1093/nar/gkw1129] [PMID]

[23] Wilkins MR, Gasteiger E, Bairoch A, Sanchez JC, Williams KL, Appel RD, et al. Protein identification and analysis tools in the ExPASy server. Methods Mol Biol. 1999; 112:531-52. [PMID]

[24] Buchan DWA, Jones DT. The PSIPRED Protein Analysis Workbench: 20 years on. Nucleic Acids Res. 2019; 47(W1):W402-7. [DOI:10.1093/nar/gkz297] [PMID]

[25] Cserzö M, Wallin E, Simon I, von Heijne G, Elofsson A. Prediction of transmembrane alpha-helices in prokaryotic membrane proteins: the dense alignment surface method. Protein Eng. 1997 Jun;10(6):673-6. doi: 10.1093/protein/10.6.673. PMID: 9278280.

[26] Kelley LA, Sternberg MJ. Protein structure prediction on the Web: A case study using the Phyre server. Nat Protoc. 2009; 4(3):363-71. [DOI:10.1038/nprot.2009.2] [PMID]

[27] Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol. 2007; 24(8):1596-9. [DOI:10.1093/molbev/msm092] [PMID]

[28] Carr PD, Ollis DL. Alpha/beta hydrolase fold: An update. Protein Pept Lett. 2009; 16(10):1137-48. [DOI:10.2174/092986609789071298] [PMID]

[29] Sun J, Zhang D, Bae DH, Sahni S, Jansson P, Zheng Y, et al. Metastasis suppressor, NDRG1, mediates its activity through signaling pathways and molecular motors. Carcinogenesis. 2013; 34(9):1943-54. [DOI:10.1093/carcin/bgt163] [PMID]

[30] Fang BA, Kovačević Ž, Park KC, Kalinowski DS, Jansson PJ, Lane DJ, et al. Molecular functions of the iron-regulated metastasis suppressor, NDRG1, and its potential as a molecular target for cancer therapy. Biochim Biophys Acta. 2014; 1845(1):1-19. [DOI:10.1016/j.bbcan.2013.11.002] [PMID]

[31] Harris NL, Loke P. Recent Advances in Type-2-Cell-Mediated Immunity: Insights from Helminth Infection. Immunity. 2017; 47(6):1024-36. [DOI:10.1016/j.immuni.2017.11.015] [PMID]

[32] Harnett W. Secretory products of helminth parasites as immunomodulators. Mol Biochem Parasitol. 2014; 195(2):130-6. [DOI:10.1016/j.molbiopara.2014.03.007] [PMID]

[33] Shapiro AD, Zhang C. The Role of NDR1 in avirulence gene-directed signaling and control of programmed cell death in arabidopsis. Plant Physiol. 2001; 127(3):1089-101. [PMID]