A *Trichinella spiralis* new born larvae-specific protein, Ts-NBL1, interacts with host’s cell vimentin

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Received: 22 October 2021 / Accepted: 2 March 2022 / Published online: 23 March 2022
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Abstract

The parasitic nematode *Trichinella* has a special relationship with its host as it has a unique intracellular location within the feeder cell which is a structure derived from skeletal muscle fiber. It has been proposed that “parakines” secreted by *Trichinella* larvae serve as messengers to implement communication between the parasite and the muscle cells through a molecular cross-talk to ensure permanent coexistence within the host. The Ts-NBL1 protein is considered to be a potential key “parakine” involved in the early invasion of the muscle fiber and its transformation into a feeder cell during *Trichinella spiralis* infection. This study used for the first time yeast two-hybrid (Y2H) technology in *Trichinella* to identify Ts-NBL1 interacting proteins. GST co-affinity purification experiments confirmed vimentin as an important interactor. The discovery of the new host proteins interacting with Ts-NBL1 will help to suggest that Ts-NBL1 contributes to participate in the capsule formation of feeder cells and provide ideas for understanding the molecular and cellular mechanisms involved in the survival of *Trichinella* in the host.

Keywords *Trichinella* · Vimentin · Proteins interaction

**Abbreviations**

| Abbreviation | Description |
|--------------|-------------|
| NC | Nurse cell |
| dpi | Days post-infection |
| NBL | Newborn larvae |

Section Editor: Peter Fischer

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**Introduction**

*Trichinella* is a genus of intracellular parasitic nematodes distributed worldwide, with the ability to infect almost all monogastric mammals including humans, birds, and reptiles. It was ranked third among the 25 most significant foodborne parasitic diseases at the European level (Bouwknegt et al. 2018). *Trichinella* genus is divided in two clades. While the species of both clades are capable of forming a feeding site named “nurse cell” (NC), they can be distinguished by the collagen capsule that surrounds it. Members of one clade produce a typical collagen-based cyst wall and are called encapsulated species. On the other hand, the collagen capsule of the other clade is poorly developed and difficult to visualize under the light microscope; therefore, it is referred to a non-encapsulated species (Xu et al. 1997).
The life cycle of *T. spiralis* is completed within a single host species and infection starts with the ingestion of infective muscle larvae followed by digestion of the protective capsule within the host stomach (Gottstein et al. 2009). The muscle larvae migrate to the small intestine where they penetrate intestinal epithelium and undergo four molts over a 30-h period to reach sexual maturity. The adults mate within the intestinal epithelium, and embryogenesis takes on 4 days post-infection (dpi). Each female releases newborn larvae (NBL) approximately 4–6 dpi (Gagliardo et al. 2002). The NBL exit the epithelium from the basolateral face, migrate through the bloodstream and lymphatic vessels, and then invade the cells of the striated muscles. The major secretory cells of the parasites, the stichocytes, increase in number through the bloodstream and lymphatic vessels, and then invade the cells of the striated muscles. The major secretory cells of the parasites, the stichocytes, increase in number from about 20 on day 1 to about 50–55 by day 19 (Despommier et al. 1998). The NBL infection of striated muscle cells induces developmental changes culminating with the formation of a novel host-parasite structure known as “nurse cell.” This is composed of a collagenous wall and cellular components that contribute to the protection of the parasite from its host immune response and participate in its long-term survival. The development of the NC is complex and it is believed that some factors produced by *Trichinella*, called “parakines,” may act as messengers to communicate with the host at the molecular level and ultimately alter the host cells’ fate to meet the parasite’s needs (Despommier 1998). Although the existence of “parakines” has been predicted on the basis of the complex interactions that occur between the mammalian cell and the parasite during NC formation, no putative “parakine” has been characterized so far. Little is known about the molecular and cellular factors and their mechanisms involved in *Trichinella* development and its survival within the cytoplasm of the NC. However, since mRNA coding for collagen is present within the NC at 9 dpi, studies have suggested that NC formation mainly begins during the NBL stage from 4 to 15 dpi (Polvere et al. 1997; Despommier 1998). Therefore, more attention should be paid on the early stage of *Trichinella* infection, as striated muscle fibers might receive parasitic signals before their invasion by NBL.

Multiple proteases have been reported in excretory/secretory products from parasitic nematodes. Their potential roles is to penetrate the host tissues, molt, and evade host innate defenses (Hewitson et al. 2009; Yang et al. 2015, p. 1; Abuzeid et al. 2020). Most of *Trichinella spiralis* proteases such as TsSerP (Trap et al. 2006) or TspSP-1.2 (Wang et al. 2013, p. 5) are excreted at all stages, but a few are stage specific such as TspSP1, excreted by the muscle larvae stichocytes (Romaris et al. 2002). Interestingly, *Trichinella spiralis* NBL1 (Ts-NBL1) was the first identified early stage newborn larvae-specific protein of *T. spiralis* (Liu et al. 2007). It has a signal peptide at the N-terminus which could designate the protein toward the secretory pathway, a conserved trypsin-like domain, and its C-terminal part is highly immunogenic (Yang et al. 2015, 2016). The early-stage-specific and secreted characteristics make Ts-NBL1 a potential key factor involved in *Trichinella* invasion and the development of nurse cell.

The aim of this study was indeed to analyze whether or not Ts-NBL1 could be linked to any interaction with cell factors particularly in the formation of the collagen capsule by checking first its belonging to the encapsulated clade or not. Our work was focused on Ts-NBL1 interactions with the host’s proteins by using yeast two-hybrid system which is widely used for screening of protein–protein interactions (Fields and Song 1989). Interaction of Ts-NBL1 with identified protein was confirmed with GST pull-down, qPCR, and co-localization by immunofluorescence labelling.

**Materials and methods**

**Phylogenic analysis and sequence alignments of Ts-NBL1 with other species of the Trichinella genus**

A homology gene analysis of Ts-NBL1, *T. spiralis* (AAR36900.1), with other *Trichinella* species was performed with sequences from *T. nativa* (KRZ256488.1), *T. britovi* (KRY56140.1), *T. nelsoni* (KRX20959.1), *T. murrelli* (KRX46800.1), T6 (KRX76874.1), T8 (KRZ92112.1), T9 (KRX65963.1), *T. patagoniensis* (KRY14817.1), *T. pseudospiralis* (AEV21546.1), *T. papuae* (KRZ79292.1), and *T. zimbabwensis* (KRZ08368.1). MEGAX and ClustalX programs were used for the construction of phylogenetic tree and the comparison of multiple sequence alignments.

**Cloning**

GenBank accession number Ts-NBL1 (AY_491941.1) and vimentin (NM_003380) coding sequence were amplified by RT-PCR from total RNAs extracted from *T. spiralis* (ISS_004) newborn larvae and human foreskin fibroblast (HFF) cells which were stored in –80 °C, respectively. DNA sequences encoding Ts-NBL1-FL (full length), Ts-NBL1-N (N-terminal, AA 29–290), Ts-NBL1-C (C-terminal, AA 291–430) (Fig. 1), and vimentin were amplified with Gateway cloning primers, 5′ ends of forward primers were fused to attB1.1 recombination sequence 5′-GGGACACCTTT GTACAAAAAGTTGGCATG-3′, while reverse primers were fused to attB2.1 recombination sequence 5′-GGGGAC AACTTTGTACAAAAGTTGGATTA-3′ (Table 1). Standard PCR was performed and PCR products were cloned into pDONR207 by an in vitro recombination-based cloning system (BP reaction, Gateway system, Invitrogen) following manufacturer’s recommendation. *Ts-NBL1* fragments
or vimentin encoding sequences were transferred by an in vitro recombination (LR reaction) from pDONR207 into pmCherry-C1, pCIneo-3xFlag, or pDEST27-GST (Caignard et al. 2013) following manufacturer’s recommendation. All recombinant constructions were transformed and amplified in Escherichia coli DH5α strain (Invitrogen).

**Cell culture and transient transfection**

All cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM, Invitrogen) supplemented with 10% heat inactivated fetal bovine serum, penicillin, and streptomycin at 37 °C with 5% CO₂. C2C12 cells (ATCC® CRL-1772™) were dispensed in 12-well plates 24 h before use. They were placed on a monolayer for stem cell growth. Cherry alone and recombinant Cherry-Ts-NBL1 vectors were transfected to C2C12 cells at 1 µg of each plasmid DNA per well. To achieve overexpression in HEK-293 T cells, Ts-NBL1 and vimentin were subcloned from pDONR207 vectors to different expression vectors as described above. GST-tag or 3xFlag-tag recombination constructions were transfected in 6-well plates at 2 × 10⁶ cells per well. Transfection was performed with jetPRIME® (Polyplus-transfection) according to manufacturer’s instructions.

**Yeast two-hybrid screening procedure**

The yeast two-hybrid procedure was performed according to a previously used protocol (Caignard et al. 2007). Briefly, Ts-NBL1-FL, Ts-NBL1-N, and Ts-NBL1-C were transferred into the Gal4-BD yeast two-hybrid vector pPC97 (Invitrogen) from pDONR207 and transformed in Y2H gold yeast strain (Clontech) according to standard procedures. The GAL4-BD-Ts-NBL1-FL, -Nter, and -Cter fusion proteins were tested for autonomous transactivation of HIS3 reporter gene and then used to screen by mating a human lung epithelial cell cDNA library (derived from A549) cloned into pDEST22 vector (Invitrogen) and already established in Y187 yeast strain (Clontech). After growing on a selective medium lacking histidine and supplemented with 80 mM 3-amino-triazole (Sigma-Aldrich), [His +] colonies were picked and maintained for extra 3 weeks to eliminate false positives. AD-cDNAs from [His +] colonies were amplified by PCR using primers that hybridize within the pDEST22 regions flanking cDNA inserts. PCR products were sequenced and analyzed by blast to identify the host proteins interacting with Ts-NBL1.

**Co-affinity purification experiments**

HEK-293 T cells (kindly provided by Dr. G. Caignard) were plated in 6-well plates (2 × 10⁶ cells per well) and 24 h later transfected with 500 ng of GST-tagged vectors and 300 ng 3xFlag-tagged vectors. Two days after transfection, HEK-293 T cells were washed in phosphate-buffered saline (PBS), and then resuspended in lysis buffer (20 mM MOPS-KOH pH 7.4, 120 mM of KCl, 0.5% Igepal, 2 mM β-Mercaptoethanol), supplemented with Complete Protease Inhibitor Cocktail (Roche). Cell lysates were incubated on ice for 20 min, and then clarified by centrifugation at 15 000 × g for 15 min. For pull-down analysis, 300 µl of each protein extraction was incubated for 2 h at 4 °C for 2 h at 4 °C.
with 35 µl of glutathione-sepharose beads (Amersham Biosciences) to purify GST-tagged proteins. Beads were washed 3 times in ice-cold lysis buffer and proteins were recovered by boiling in denaturing loading buffer (Invitrogen).

**Western blot analysis**

The purified complexes and protein extracts were analyzed by 12% SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred on a nitrocellulose membrane. Proteins were detected using standard immunoblotting techniques. GST-tagged proteins were detected with a rabbit polyclonal anti-GST antibody (Sigma-Aldrich) and a HRP-conjugated anti-rabbit secondary antibody (Invitrogen). 3xFlag-tagged proteins were detected with a mouse monoclonal HRP-conjugated anti-3xFlag antibody (Sigma-Aldrich).

**Quantitative PCR analysis**

HEK-293 T cells were plated in 24-well plates (2 × 10^5 cells per well). Twenty-four hours later, 0.5 µg/well pcNeo-3xFlag vector alone or fused with Ts-NBL1 was transfected with jetPRIME® (Polyplus transfection). Two days after transfection, cells were recovered in PBS and total RNA was isolated by RNAqueous®-Micro Kit (Invitrogen) according to manufacturer’s instructions. RNA yields were evaluated using a NanoDrop spectrophotometer. cDNA synthesis was achieved by starting from 2 µg of total RNA following manufacturer’s recommendation. Quantitative PCR was performed to measure transcription levels for a 100 bp vimentin fragment (primers: Fwd: 5′-AGGCAAAGCAGAGTCCACTGA-3′; Rev: 5′-ATCTGGCGTCCAGGACTCAT-3′) and a 131 bp fragment of the GAPDH housekeeping gene. QPCR was performed using the LightCycler 480 (Roche Diagnostics). The PCR cycle was an initial incubation of 10 min at 95 °C, 40 amplification cycles of 10 s at 95 °C, of 10 s at 60 °C, and of 30 s at 72 °C, during which SYBR Green fluorescence levels were collected. Results were normalized using expression levels of the housekeeping gene.

**Immunostaining and subcellular localization**

To perform subcellular localization experiments, 12-well plates containing coverslips were seeded with 1 × 10^5 C2C12 cells per well. Twenty-four hours later, Cherry alone and Cherry-Ts-NBL1 vectors were transfected by jetPRIME® following manufacturer’s recommendation. Twenty-four hours after transfection, cells were incubated with 4% PBS-PFA for 20 min, and then washed 2 times with PBS and incubated for 45 min at 37 °C with blocking solution (5%BSA, 0.3%Triton® X-100). After removing blocking solution, cells were incubated with rabbit polyclonal anti-vimentin antibody (Invitrogen), washed and stained with anti-rabbit Alexa Fluor-488-conjugated secondary antibody (Invitrogen). Finally, cells were incubated for 5 min in a PBS containing DAPI (4′-6-Diamidino-2-phenylindole) at 10 µg/ml and covered with aqueous mounting medium. Image acquisitions were performed using a Leica DMI 8 confocal microscope.

**Statistical analysis**

All the data were shown as arithmetic means ± standard deviation (SD) and performed with Kruskal–Wallis test followed by Dunn’s post-test. The statistical test was regarded significant as follows: *p < 0.05; **p < 0.01.

**Results**

**Phylogenic analysis and sequence alignments of Ts-NBL1**

As shown in the phylogenetic tree generated with Ts-NBL1 and its orthologues (Fig. 2), the sequences are grouped as the two branches corresponding to on one side the encapsulated and on the other side the non-encapsulated *Trichinella* species. Compared with encapsulated clade (black squares), the species in non-encapsulated (red squares) are a monophyletic group with bootstrap value of 100. Also, a homology comparison of Ts-NBL1 and other orthologues in the genus *Trichinella* was determined among these sequences (Fig. 3). Interestingly, Ts-NBL1 homologous genes show different identities between encapsulated and non-encapsulated species. In encapsulated species (*T. nativa, T. britovi, T. murelli, T. nelsoni, T. patagoniensis*, and genotype T6, T8, T9), there are more than 90% identity with Ts-NBL1, while with non-encapsulated species *T. pseudospiralis, T. papuae, and T. zimbawensis*, the identity drop to 88%, 86%, and 85% respectively.

**Y2H screening of Ts-NBL1 potential interacting proteins**

To screen the host proteins interacting with Ts-NBL1, Y187 yeast cells expressing a human cDNA library were used to mate with Y2H gold yeast strain expressing *Ts-NBL1* fragments, as described above. After 6 days of culture on selective medium, [His +] colonies were picked, purified during 3 weeks by culturing on selective medium. A total of 193 cultured colonies allowed identifying *Ts-NBL1*-C binding...
partners after elimination of false positives (Fig. S1a). These 193 colonies were amplified by PCR from zymolase-treated yeast colonies using primers that hybridize within the pDEST22 regions flanking cDNA inserts (Fig. S1b). PCR products were sequenced, and cellular interactors were identified by blast analysis. A total of 193 colonies corresponded to 20 potential interacting proteins are listed in this table according to the number of colonies from most to least (Table 2). Thirty-one colonies corresponded to undescribed proteins, which are not shown in this table. Gene ontology analysis indicated that these interactants participate in putative signaling pathway and biological processes (Fig. S2), such as participating in caspase cascade in apoptosis (VIM, KRT18, LMNB1), striated muscle contraction (VIM), positive regulation of cell cycle G2/M phase transition (NPM1), and positive regulation of collagen biosynthetic process (VIM). The analysis of the function, combined with the number of positive colonies, led to the consideration of vimentin as an important interactant.

**Ts-NBL1 interacts with vimentin**

To verify the interactions between Ts-NBL1 and vimentin in mammalian cells, Ts-NBL1 fused with GST-tag and vimentin fused with 3xFlag-tag were co-expressed in HEK-293 T cells and purified with glutathione-sepharose beads. Expression of each of these proteins in HEK-293 T cells was confirmed by detecting different expression tags in cell lysates. Pull-down samples were collected and detected 3xFlag-tag to assess protein interactions. Since negative control GST vector alone did not show detectable products (Fig. 4), these results indicated that vimentin was able to interact with Ts-NBL1.

**Ts-NBL1 up-regulates vimentin mRNA expression**

In order to determine the effect of Ts-NBL1 on vimentin expression, Ts-NBL1 was over expressed in HEK-293 T cells to detect vimentin mRNA expression. After normalizing with GAPDH expression, vimentin transcription level was evaluated. Compared to the control group, Ts-NBL1 transfection significantly increased the expression of vimentin with a twofold increase (Fig. 5a).

**Immunostaining and subcellular co-localization**

To identify the subcellular localization of Ts-NBL1 in cells and whether it co-localized with vimentin, Ts-NBL1 was expressed in C2C12 cells by reconstruction Cherry plasmids transfection (Fig. 5b). Vimentin was distributed around the cell nucleus and radiated towards the plasma membrane, participating in the cytoskeleton without any cell changes. Ts-NBL1 exhibited a perinuclear distribution radiating towards the periphery of the cytoplasm. A co-location of Ts-NBL1 and vimentin was demonstrated, as both signals have fused the same cell locations.

**Discussion**

It was important to compare the genomic sequences of NBL1 between the two different clades of *Trichinella* (encapsulated versus non-encapsulated) as this protein, which is expressed at early stage of development, seems to be essential for understanding the mechanisms of interaction of this parasite with its host and in particular in the establishment of the parasitic nurse cell. In this way, the comparative study of the sequences shown the evolution of the sequences between the different species of the genus *Trichinella*. A phylogenetic

![Phylogenetic tree constructed by MEGA.](attachment:tree.png)
analysis of *Trichinella* has shown that encapsulated and non-encapsulated *Trichinella* taxa diverged from their most recent common ancestor ∼21 million years ago, with taxon diversifications happening ∼10−7 million years ago (Korhonen et al. 2016). Interestingly, the NBL1 sequences are also organized into two groups where encapsulated and non-encapsulated *Trichinella* species are distributed. A strong link is thus confirmed between the NBL1 sequences

**Table 2** *Ts*-NBL1 potential interacting proteins screened by Y2H

| Protein names                                      | Abb   | Accession N° | Colonies |
|---------------------------------------------------|-------|--------------|----------|
| Endogenous Retrovirus Group K3 Member 1           | ERVK3-1 | ENSP00000489180 | 56       |
| Potassium Channel Tetramerization Domain Containing 10 | KCTD10 | ENSP00000441672 | 29       |
| Vimentin                                          | VIM   | ENSP00000435613 | 25       |
| Mitochondrial Assembly Of Ribosomal Large Subunit 1 | MALSU1 | ENSP00000419370 | 10       |
| Nucleophosmin                                     | NPM1  | ENSP00000377408 | 9        |
| EF-hand Calcium Binding Domain 14                 | EFCAB14 | ENSP00000361001 | 5        |
| KIAA1549 Like                                     | KIAA1549L | ENSP00000433481 | 4        |
| Kinesin Family Member C3                          | KIFC3 | ENSP00000458009 | 4        |
| Diacylglycerol Kinase Delta                       | DGKD  | ENSP00000386455 | 3        |
| Envolplakin                                       | EVPL  | ENSP00000301607 | 3        |
| E1A Binding Protein P400                          | EP400 | ENSP00000331737 | 2        |
| Heterogeneous Nuclear Ribonucleoprotein U Like 1  | HNRNPUL1 | ENSP00000473178 | 2        |
| Keratin 18                                        | KRT18 | ENSP00000447278 | 2        |
| Lamin B1                                          | LMB1  | ENSP00000378761 | 2        |
| Caspase 8 Associated Protein 2                     | CASP8AP2 | ENSP00000478179 | 1        |
| Potassium Channel Tetramerization Domain Containing 11 | KCTD11 | ENSP00000328352 | 1        |
| Keratin 8                                         | KRT8  | ENSP00000447881 | 1        |
| MIF4G Domain Containing                           | MIF4GD | ENSP00000484245 | 1        |
| Ribosomal Protein L39                             | RPL39 | ENSP00000355315 | 1        |
| Tripartite Motif Containing 23                    | TRIM23 | ENSP00000274327 | 1        |
and the membership of one of the two clades. Differences between encapsulated and non-encapsulated species could explain some differences on the nurse cell (NC) formation like the thick/thin collagen expressed surrounding the cell. Unveiling the function of T3-NBL1 could hold one of the key to understand the interactions of *Trichinella* with its host muscle cell and its differentiation as nurse cell structure.

In the present study, Y2H screening was used to identify host proteins interacting with T3-NBL1 to understand the molecular and/or cellular mechanisms by which cellular pathways T3-NBL1 works in the cell. A total of 193 interacting colonies corresponding to 20 proteins from a human cDNA library were identified as potential interacting proteins of C-terminal part of T3-NBL1. These potential interacting proteins are involved in many of the same biological pathways, such as the apoptotic process, cell proliferation, cell differentiation, and signal transduction. Among these potential interactants, we focused on vimentin, which was found in a large number of colonies. Vimentin is the major intermediate filaments protein found in mesenchymal cells (Eriksson et al. 2009) and also as a marker of regeneration in muscle cells (Gallanti et al. 1992). Moreover, growing evidence suggests that vimentin is involved in a series of metabolic, signaling, and regulatory processes that are not related solely to mechanical function (Ivaska et al. 2007; Kwak et al. 2012; Zhang and Stefanovic 2016).

For the first time, our study identified the reliable interaction between vimentin and T3-NBL1 by co-affinity assay in vitro. Moreover, this interaction was confirmed by GST pull-down approach. Immunofluorescence results indicated that T3-NBL1 displayed high intensity, around the nucleus with a co-localization with vimentin, at the cytoplasmic level. Many cellular signaling pathways are altered in various physiological conditions, especially reactions involving proteases (Singhirunnusorn et al. 2010).
2005). Although we are currently unable to determine whether this interaction was related to the serine protease activity of Ts-NBL1, the model we use was to study the co-localization of Ts-NBL1 at the 24-h fixed point of transfection. It could be further captured by a lifetime image instead of at a fixed point to figure out how long the interaction lasts or whether the process of interaction is transient.

The transfection of Ts-NBL1 significantly upregulated the expression of vimentin with a twofold increase. This major result in the knowledge of Trichinella interaction with its host cells could explain the impact on collagen expression for the nurse cells construction. Indeed, studies have shown that vimentin interacts with LARP6, which is involved in the regulation of collagen to stabilize collagen mRNA. The stabilization of collagen mRNA is the main mechanism of high collagen expression. Vimentin knockout fibroblasts produce reduced amounts of type I collagen due to decreased stability of collagen mRNA (Challa and Branko 2011). On the other hand, newborn larvae invade muscle cells and can cause mechanical damage and induce collagen production in surrounding cells, which accumulates in response to tissue injury. Vimentin is required for this accumulation and have been reported to mediate the early signaling process of tissue repair (Cheng et al. 2016; LeBert et al. 2018). We proposed that vimentin contributes to the collagen synthesis in the infected cells. We demonstrated that vimentin binds to Ts-NBL1 and also contributes to Ts-NBL1-dependent upregulation of vimentin, which may affect accumulation of collagen around nurse cell.

Vimentin is a marker in the process of muscle cell regeneration (Bornemann and Schmalbruch 1992). Vimentin and the muscle-specific protein desmin belong to the same assembly group and exhibit a rather high amino acid sequence identity (Bär et al. 2004). While desmin is only expressed at low levels during the early stage of muscle cell development, it increases as the cell approaches terminal differentiation. In other words, vimentin was detected in early developing muscle, but not in fully differentiated muscle (Vater et al. 1994; Vaittinen et al. 2001; Güttchtes et al. 2015). However, the muscle cells infected by T. spiralis undergo dedifferentiation at the beginning, which provides more chance for the interaction of Ts-NBL1 and vimentin. It seems like a strategy of the parasite in this process: Ts-NBL1 interacts with vimentin even upregulating the expression of vimentin to promote the cell’s destined fate to re-differentiate into NC. It has been reported that Trichinella can significantly upregulate vimentin gene expression in muscle cells 23 dpi, which is more noticeable with T. spiralis than T. pseudospiralis (Wu et al. 2008). This may be due to the differences in NC formation induced by T. spiralis (encapsulated) and T. pseudospiralis (non-encapsulated). NC formation in T. spiralis involves the fusion of transformed infected muscle cells and misdifferentiated satellite cells, whereas in T. pseudospiralis, the satellite cells are activated but never fuse to the nurse cell cytoplasm, resulting in incomplete cysts formation (Wu et al. 2001; Boonmars et al. 2004). Ts-NBL1 could thus upregulate vimentin expression in non-muscle cells, and whether the same phenomenon occurs in muscle cells remains to be confirmed. As we have an ethical duty to reduce the use of animal experiments, ex vivo model allowing the parasitic invasion of differentiated muscle cells will be needed to further identify the interaction that occurs in the actual infection of Trichinella.

Our research described for the first time the interaction of a T. spiralis stage-specific protein with a protein of the cell cytoskeleton. It is thus proposed that T. spiralis uses NBL1 as a secreted signaling molecules to carry out its own developmental programs like all the mammalian intercellular communication systems (Despommier 1998). In this way, NBL1 could be considered “parakines.” Our study would thus be the first identification and characterization of the communication between the mammalian cell and the worm during nurse cell formation. The importance of this interaction Ts-NBL1 /vimentin is all the more important as the parasitic protein is expressed at an early and invasive stage of the parasite’s live cycle, which coincides with the installation in the muscle cell.

An overexpression of vimentin could explain a collagen production mechanism that contributes to the constitution of a NC with a protective collagen capsule as described for encapsulated species.

In summary, the identification of key Ts-NBL1 protein interactions in the early stage of T. spiralis provides insight into the molecular and cellular mechanisms involved in Trichinella survival in host cells. This contributes to the understanding of nurse cell formation and high collagen expression in the encapsulated clade.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00436-022-07479-7.

Author contribution The study was conceived and designed by AW, GK, and IV. Experiments were performed by AW, AH, GC, DV, EH, and GK and data were analyzed and discussed by AW, AH, GC, DV, EH, GK, and IV. AW wrote the manuscript with GK and IV. It was then improved by XL, ML, PB, and GC. All authors read and approved the final manuscript.
Funding This work was supported by the China Scholarship Council, OIE Collaborating Centres for Zoonotic foodborne parasites (French and Chinese), DIM1HEALTH Région Île-de-France grant for the confocal microscope, and Laboratoire d’Excellence Integrative Biology of Emerging Infectious Diseases grant ANR-10-LABX-62-IBEID.

Declarations

Conflict of interests The authors declare no competing interests.

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