Biological Times

Volume No. 4 | Issue No. 2 | Pages 1-3

Role of siRNA to knockdown the Genes in Trichinella spiralis and Future Perspectives

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ABSTRACT

Trichinella spiralis is a multicellular parasite that has zoonotic importance and causes trichinellosis in humans. It is transmitted from animals to humans through the eating of raw or poorly cooked meat contaminated with muscle larvae. Due to this parasite, meat food safety is disturbed. Initially, anthelmintic drugs are used for their control, but these have certain limitations. So, there is an urgent need for the development of new methods to control *T. spiralis* infection. Small interfering RNA is synthesized in the laboratory artificially. These are used in molecular biology to evaluate the function and characteristics of different genes. This technique was employed to find novel candidate targets for the development of vaccines. The whole sequence of *T. spiralis* is completed but the role of many genes is still unclear. RNAi is used to investigate different genes.

Keywords: siRNA, Gene knockdown, Host–nematode interactions, *Trichinella spiralis*

To cite this article: Munir F, H Khan, A Shakoor, S Nasir & ZUD Sindhu. Role of siRNA to knockdown the Genes in Trichinella spiralis and Future Perspectives. Biological Times. 2025. February 4(2): 1-3.

Introduction

Trichinella spiralis (*T. spiralis*) is an enzootic nematode that is responsible for the food-borne disease trichinellosis [1]. People acquire infection by eating raw or undercooked meat containing infective larvae. This parasite is not only responsible for public health concerns but also poses serious issues to meat food safety. This is a zoonotic parasite and its transmission to humans from pork is more common in developing countries [2,3]. It is diagnosed with more than 150 mammalian species around the globe [2]. When humans ingest meat containing the encapsulated muscle larvae (EML), they are liberated in the stomach with the help of proteolytic enzymes. Encapsulated muscle larvae molt into intestinal infectious larvae 1 (IIL1) in the intestine. These L1 then invade the epithelial cells of the intestine and after four consecutive moltings grow into adult worms [3,4]. The life cycle of *T. spiralis* is unique because its developmental stages occur within the same host [7]. In the world, *T. spiralis* is ranked as the seventh most crucial zoonotic food-borne disease. Due to many *T. spiralis* hosts and the lack of vaccination as a prophylactic measure, its control is difficult. [2] Despite having many animal reservoirs and a lack of Anti-*T. spiralis* vaccines, derivatives of benzimidazole such as mebendazole, albendazole, thiabendazole, and flubendazole are commonly used for the treatment of trichinellosis. The effectiveness of anthelmintics greatly depends upon the time of administration as these may be ineffective in case of chronic infection [3]. However, the widespread use of anthelmintic drugs has resulted in the development of resistance and people demand to restrict the use of these drugs as their residues appear in animal products such as meat and milk and limit the control of *T. spiralis* infection [5]. Certain anthelmintics (mebendazole) in rats are teratogenic and contraindicated for children under two years of age and pregnant women [3]. For the control of trichinellosis, alternative methods such as management protocol improvement, development of vaccines, and new drugs are needed urgently [5].

For the control of parasitic infection, a better understanding of the parasite's biology is necessary for the recognition of new, novel, and effective targets of control. Small interfering RNA (siRNA) is also known as silencing RNA which is double-stranded RNA and has a gene sequence of 21-25 nucleotides. In *C. elegans* these small RNAs were identified [6]. It has an important role as a regulator in post-transcriptional gene expression. siRNAs are non-coding and short RNAs responsible for various regulatory functions such as having a role in immunity, cancer, and the development of organs [5]. These siRNAs are artificially synthesized and studied in host-parasite interaction. They regulate gene expression negatively. The interest of scientists to study siRNA synthesized from double-stranded RNA for silencing the expression of the gene and serving as an effective tool for the study and identification of gene functions has increased over the last decade [1,2].

RNA interference technique is experimentally studied for the control of nematode parasites. Their importance was first demonstrated in *Caenorhabditis elegans* (*C. elegans*) which are free-living roundworms. The non-coding RNAs were identified in *C. elegans* such as lin-4 and let-7 which are responsible for the accurate development of larval stages from L2 to L3 and from L4 to adult worms. The family mir-36 is important for the development of an embryo, the family mir-51 is necessary for the attachment of the pharynx during the development of an embryo (embryogenesis), and the mir-58 family has an essential role in the regulation of growth, locomotion, and the arrested dauer stage larvae development [5]. Libraries of small RNA from different developmental stages of helminths are used to generate information about the siRNA of helminth parasites. This method is used to characterize and recognize siRNA in different nematodes, tapeworms, and flukes. This method is also used to characterize siRNA in *H. contortus, A. suum, Trichinella spiralis, Burgia species, Taenia saginata, Angiostrongylus cantonesis, Schistosomes,* and *Echinococcus.* Many siRNA are unique to only one species. These findings suggest that small transferring RNA may have an essential role in the transmission of parasites and their ability to habituate different environments. The regulatory pathways, genes, and siRNA are of interest for the development of novel targets of prevention and control [2,5]. Interfering RNA was used to find the specific targets for the development of vaccines and drugs in parasites such as *Clonorchis sinensis, Setaria digitata,* and *Brugia malayi* [3]. Many types of siRNAs have been identified in *C. elegans, Schistosoma japonicum, Drosophila melanogaster, Arabidopsis thaliana,* fungi, and mice [1].

The short interfering RNA can be used to silence and suppress certain genes that are essential to produce proteins necessary in the developmental processes, molting, and life cycle of *T. spiralis* to decrease the burden of parasite invasion in humans and other mammals [2,3]. Interfering RNA has been used to understand the functioning of different genes in different parasites such as the heat-stable calcium-regulated and collagen type V proteins in *Schistosoma japonicum* and *Clonorchis sinensis* enolase was evaluated. Interfering RNA was also successfully evaluated in *Nippostrongylus brasiliensis* nematode [7].

Function of Small interfering RNAs (siRNAs)

The RNA interference (RNAi) method has many important functions. They are important in the regulation of post-transcriptional genes. They are involved in the negative regulation of gene expression. RNAi technique is used to understand the functions of different genes in parasites due to their capability to suppress a specific sequence of transcriptional genes [2,3]. The siRNA has an essential regulatory role in fundamental processes of cells such as differentiation of cells, proliferation, apoptosis, metabolism, and stress response. The siRNA derived through transposable elements (TE-derived siRNA) represents transposon activity in the germline cells and functions to maintain the stability of the genome while the siRNA derived through natural antisense transcripts (NAT-derived siRNA) becomes more active in somatic cells [1]. The life cycle of helminths consists of many developmental stages. siRNA has an important function in the development of parasites. The small interfering RNAs act as mediators of interaction between the host and parasite. siRNAs are responsible for the interaction of

parasite nematodes with the host environment. In pathologic conditions in humans such as cancer or tissue damage, changes in the siRNA expression occur and can be detected in urine, plasma, and blood samples. Therefore, these can be used as a marker for the identification of many diseases [3]. In recent parasitic studies, it is reported that siRNA of helminths was detected in the plasma or serum of infected hosts. However, it is not recognized whether this siRNA is from live or dead parasites. RNA-interfering genes are highly sensitive and specific and may serve as the best candidates as a biomarker for the diagnosis of parasitic infections [6]. Due to the high specificity of siRNA, it is possible to distinguish between the parasite's egg-positive and negative individuals. A serine protease 1.2 (TsSP1.2) of *T. spiralis* was recognized with the help of immunoproteomics in the excretory-secretory (ES) protein of muscle larvae and infectious intestinal larvae. The protein and TsSP1.2 mRNA were silenced by using Interfering RNA methods [3,6].

3. siRNAs in host–nematode parasite interactions

The siRNA was first studied in the model nematode *C. elegans.* The mechanism (refer to Figure 1) of siRNA action in *C. elegans* involves the interaction of primary siRNA with the Argonaute protein RDE-1 to recognize and cleave the homologous sequence of mRNA. Secondary siRNA amplifies this response which is produced by RNA-directed RNA polymerase EGO-1 or RRF-1 [5,6]. siRNAs direct specific gene silencing when bound with the sequence complementary to their whole length [6]. Excretory-secretory (ES) proteins are involved in long-lasting interaction between the host and the parasite immunologically. siRNA. Major histocompatibility complex (MHC) class I and II molecules are involved in immune responses through antigen processing and presentation. In B-cells, monocytes, macrophages, and dendritic cells, the expression of gamma interferon-inducible lysosome thiol reductase (GILT) occurs. It is involved in the restriction of MHC-II. Major histocompatibility complex-II speeds up the breakdown of the disulfide bond and enhances the cleavage process through cellular proteases [7]. siRNA can be used to mask the expression of GILT so that the worm (nematode) burden can be decreased.

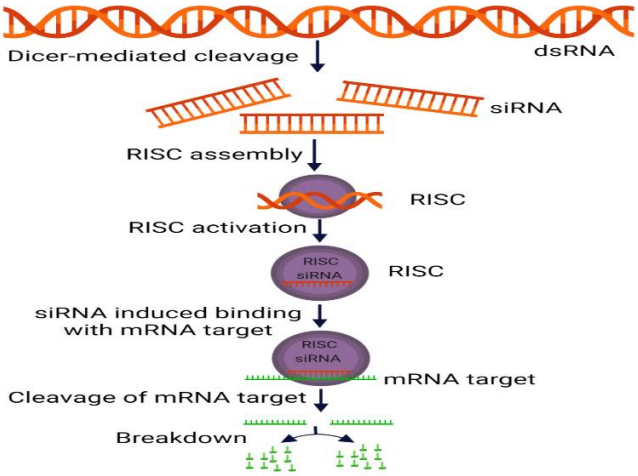

Fig. 1: Mechanism of siRNA action

Si-RNA action against *Trichinella spiralis*

The nematodes which are zoonotically important such as *T. spiralis*, the RNAi technique was used to understand the function of genes that encode nudix hydrolase and paramyosin (proteins) required for the infectivity and viability of the parasites [3,6,8,9]. With the help of siRNA, *T. spiralis* GILT protein was studied in nematode *T. spiralis.* It was found that the molecular characteristics of Tsp-GILT are the same as those of known GILT proteins. In a study, it was found that they may possess the same function immunologically. The reduction of IgG into the H and L chains could also occur with Tsp-GILT. In a recent study, it was evaluated that the use of siRNA resulted in the reduction of adult worm size. The effect of the siRNA-mediated knockdown was also studied on the development of *T. spiralis*. It was found that the Tsp-GILT suppression did not result in a significant effect on the survival of muscle larvae, growth, and reproduction of adult worms. However, there was a significant decrease in the number of next generation (nurse cells) [9]. The stage of newborn larvae occurs inside the host organism. In this stage, the expression of Tsp-GILT is more as compared to other stages. Finally, the result was concluded that Tsp-GILT may play an important function in the interaction between parasite and host [8]. Serpin-type serine protease inhibitors of *T. spiralis* (TsSPIs) were identified in muscle larvae, newborn larvae, and adult worms of *T. spiralis*. Their highest expression is in the muscle larval stage of development. These are essential in the larval invasion and survival of parasites inside the host environment infected by *T. spiralis.* The silencing of TsSPI mRNA in *T. spiralis* with the help of the RNAi technique has resulted in a decreased survival-ability and infectivity of larvae in the host. TsSPI may be responsible for the regulation of *T. spiralis* and the host directly. Further, it was found that they do not affect reproduction, growth, fecundity, and survival rate [8,10]. The *T. spiralis* paramyosin (Ts-pmy) function in the development of growth and viability was first confirmed with the help of siRNA silencing transcription of Ts-pmy mRNA. The role of Glutaminase (GLS) gene was assessed in *T. spiralis* with the help of siRNA silencing. These are important in the glutamine-dependent acid resistance system. *T. spiralis* GLS (Ts-GLS) is expressed in the muscle larval stage of the development of the parasite. Glutaminase is important in the survival of *T. spiralis* and is expressed in the epidermis of muscle larvae. These can serve as a potential target for the development of vaccines [11].

Future perspectives

The sequence of the complete genome of *T. spiralis* is known but the biological characteristics and function of many genes are still not clearly understood. With the help of siRNA methods, we can evaluate the function of different genes of parasites including *T. spiralis*. CRISPR-Cas9 is a new technique used to evaluate and edit the genome of eukaryotes and protozoa, but it is difficult to perform in multicellular organisms. The RNAi technique can be used to investigate the functions of genes in multicellular, including *T. spiralis* with great ease [10]. The most important weapon against parasite invasion is chemotherapy but there is increased development of resistance in the parasites to the drugs used against them [12]. siRNA technique can be employed to identify novel target candidates for the development of vaccines and drugs to control and prevent various diseases [11].

Conclusion

RNA interfering is a technique used to investigate the biological characteristics and functions of different genes. In this method, we use small interfering RNA to silence and suppress the expression of a specific gene in vivo as well as in vitro. This can also be used in multicellular organisms easily and proved to be an effective tool for the identification of the various roles of genes and to identify novel targets for the development of vaccines. In molecular biology, it has great importance.

Table 1: siRNA and their function

siRNA name	Developmental stage	Nematode species	Function	Reference
siRNA 534	Muscle stage larvae	*Trichinella spiralis*	Suppress *Trichinella spiralis* serene protease 1.2 (TsSP1.2) mRNA, and protein expression in muscle and intestinal infectious larvae (IIL1)	[10]
siRNA 156	Muscle stage larvae	*T. spiralis*	Suppress *Trichinella spiralis* serene protease (TsSP) mRNA and protein expression in muscle larvae	[9]
siRNA153	Adult worms, newborn larvae, and Muscle larvae	*T. spiralis*	Suppress *T. spiralis* serpine-type serine protease inhibitors essential for the invasion of larvae	[5]
siRNA479	Adult worms, newborn larvae, and Muscle larvae	*T. spiralis*	Suppress *T. spiralis* serpine-type serine protease inhibitors essential for the invasion of larvae	[5]
siRNA986	Adult worms, newborn larvae, and Muscle larvae	*T. spiralis*	Suppress *T. spiralis* serpine-type serine protease inhibitors (TsSPIs) essential for the invasion of larvae	[5]
siRNA 881	Adult worms, newborn larvae, and Muscle larvae	*T. spiralis*	Suppress *T. spiralis* Glutaminase (TsGLS) which plays a crucial role in the glutamine-dependent acid resistance system	[11]

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