Abstract

Setaria digitata is a parasitic nematodes residing in the abdominal cavity of ungulates and their natural host is cattle to which *S. digitata* is non-pathogenic. When infective L3 larvae stage enters into non-permissive hosts such as goat, sheep or horse could cause cerebrospinal nematodiasis. Human can also be infected by *S.digitata* via mosquito bites causing abscesses, allergic reactions, enlarged lymph nodes, eye lesions etc., due to harboring microfilaria by human.

Small interference RNA(siRNA) mediated RNA interference is a powerful technique to study the function of genes that organism genome encodes and this has also been used to unravel function of genes of nematodes. SdNP is *a* novel parasitic nematode protein of *S. digitata* expressed at all stages of lifecycle and abundantly found in longitudinal muscle, reproductive system and embryos. In this study, siRNA-mediated silencing of the *SdNP* expression was carried out to study the function of the latter following confirmation of *SdNP* expression in adult nematodes by RT-PCR. In doing so, dsRNA was generated by PCR using cloned *SdNP* with four set of primers. This generated dsRNAs were cleaved to obtain 21-23bP siRNA fragments using shortcut RNAse III prior to labeling with Cy3, which was then used to treat adult worms (40µg/ml) for 3 hours/ day for 3 days. siRNA treatment was carried out in culture medium containing in RPMI 30 µg/ml Streptomycin,2.5µg/ml Amphotericin B in a CO_2 incubator in the presence of 5% CO_2 and at 37 ^{O}C without Fetal calf serum. The treated and non treated worms were preserved in 4% formaldehyde in PBS and embedded in paraffin block. The paraffin embedded tissues were cut using microtome and subjected to immunohistochemical staining using polyclonal antibody raised against SdNP. Permanent slides were observed using light microscope. The observation of sections under the fluorescence microscope indicated successful up taking of Cy3 labeled siRNAs by adult worms and the clear motility reduction was observed in siRNA treated group. The comparison of immunohistochemical staining of the section treated and untreated indicated down regulation of SdNP expression. Therefore taking these outcomes, it can be concluded that RNAi technology could be used successfully to down regulate expression of target genes in *S. digitata*.