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L3 Gene: Exploring the Presence in Syrian Strain of *Leishmania Tropica* Genome

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Abstract

Cutaneous leishmaniasis represents a serious health problem in Syria, this problem has become noticeably aggravated after the civil war in the country. *Leishmania tropica* parasite is the main cause for the cutaneous leishmaniasis in Syria. In order to control the disease, we need an effective vaccine against leishmania parasite. DNA vaccination remains one of the favorable approaches that have been used to face cutaneous leishmaniasis. Ribosomal protein L3 is responsible for important roles in Leishmania parasite life. DNA vaccine based on L3 gene has been used against infections by many species of Leishmania parasite but *leishmania tropica* parasite, so this gene represents a good candidate for DNA vaccine construction. But the presence of this gene has not yet been demonstrated in the genome of *Leishmania tropica*. So, this study aims to explore the presence and the expression of this gene in Syrian strain of Leishmania tropica promastigotes. The DNA and RNA were extracted from Syrian strain of *Leishmania tropica* parasites, the cDNA was made, the primers for genes' amplification were designed manually, the conditions of the PCR were optimized and cDNA was used as a template. Full-length double-stranded sequence analysis was done. The Extracted genomic DNA and total RNA were with high degree of integrity and purity. L3 PCR products were shown by gel electrophoresis, on the level of DNA and cDNA, only one band goes to L3 gene, and their sizes were approximately 1260 bp. The sequence of the cDNA-L3 gene was determined and published in GenBank, according to the sequence the exact size of the gene was 1260 bp. Expression was also proven at the level of cDNA. Ribosomal protein L3 gene is a part of Syrian strain of L. tropica, the expression of this gene has been proven. The sequence of the cDNA of ribosomal protein L3 gene has been defined and submitted to the Genbank with accession number NO. MN495878.1.

Keywords: *Leishmania tropica*; ribosomal protein L3 gene; Syria.

Introduction

Leishmaniasis represents a serious issue in developing countries, the estimated number of cases reaches up to 0.9 to 1.3 million case every year. About ninety tropical and subtropical countries have leishmaniasis as a plague disease, with approximately 350 million people who are in the danger circle to get the disease. There are three clinical subtypes of leishmaniasis: cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis, and visceral leishmaniasis. cutaneous leishmaniasis (CL) is the most widespread subtype of the disease caused by unicellular parasite and transmitted by sandfly. *L. tropica* is the most involved cause of cutaneous leishmaniasis [1]. Worldwide, there are annually 1 to 1.5 new cutaneous leishmaniasis cases. Syria is one of the 10 countries that hold more than 85% of registered cutaneous leishmaniasis cases in the world [2].

The treatment of leishmaniasis nowadays depends on the use of chemotherapy agents which lie in many obstacles such as high cost, dangerous and unpleasant side effects, high toxicity, the development of dug resistance especially in endemic areas. So, the invention of a vaccine against leishmania represents one of the most promising tools to control and element the disease [3].

DNA vaccination technology involved the use of DNA plasmids that are recombinant with the target genes, these recombinant plasmids eventually enter the nucleus of the mammalian cells where the target genes are expressed into proteins that are released to the cytoplasm, these expressed antigens are presented to the immune cells by the mean of MHCI or MHCII

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molecules. Innate immunity is also being stimulated by DNA vaccine which represents an essential step in order to elicit appropriate cellular immune responses against the MHC-presented antigens, so, DNA vaccination strategy guarantees the stimulation of the required humoral and cellular immune responses [4].

Ribosomal proteins family is one of the most conserved protein families in the world. Ribosomal proteins have the appropriate immunogenic characterizations and are called pan antigens. These pan antigens represent the most suitable and the strongest candidates for vaccine design due to their ability to trigger specific and strong immune responses [5]. Ribosomal proteins had succeeded to elicit such a strong and protective immune response when they are used as DNA vaccines at many bacteria [6, 7] and parasites [8]. Recombinant ribosomal proteins L3 or L5 with the TH1-inducing adjuvant had elicited protective responses against CL caused by L. major [9].

Ribosome is a universal, essential and the most complex cellular ribonucleoprotein particle in the cell, the ribosome catalyzed the decoding of the genetic information. The ribosome consists of a large and a small subunit, ribosomal protein L3 is a structural component of the large subunit of the ribosome. The large ribosomal subunit is responsible for the catalysis of peptide bond formation between aminoacyl-tRNAs and peptidyl-tRNAs during the elongation step of protein synthesis, and for the hydrolysis of peptidyl-tRNA during translation termination. Both reactions take place in the peptidyltransferase (PTase) centre of the ribosome. Biochemical studies demonstrate the crucial role of ribosomal protein L3 in the ribosomal catalytic activity [10].

So, in the regard of the crucial roles of the ribosomal protein L3 in leishmania parasites life and the capability of this panantigen to induce perfect immune response against many bacteria and parasites included leishmania, we conclude that the ribosomal protein L3 and its gene represent good candidates that cold be use to design vaccine against leishmania. In this study, we decide to investigate the existence of ribosomal protein L3 in LCED Syrian 01 strain of *L. tropica* genome, because neither the presence nor the sequencing have been done yet for this gene. This work was a first step needed to be followed by the examination of L3 gene as a DNA vaccine against *L. tropica*, parasite infection which is the main parasite that is responsible for the cutaneous leishmaniasis cases in Syria. This present systematic study is the first to investigate the presence of this gene in LCED Syrian 01 strain of *L. tropica* genome, to prove if there is an expression of this gene in these parasites and to sequence it.

Materials and methods

Parasite culture

Leishmania Center for Epidemiological and Biological Studies, Damascus University had provided LCEB-Syrian Strain 01 of *Leishmania tropica*. RPMI -1640 medium (Lonza-USA) supplemented with 5% fetal calf serum was used to grow Promastigotes.

Genomic DNA extraction

DNA extraction kit (Promega, USA) was used to extract the Leishmania genomic DNA from the promastigotes according to the manufacturer's instructions, the extracted DNA was electrophoresed on 1% agarose gel.

Total RNA extraction and cDNA synthesis

5ml of RPMI medium containing promastigotes was centrifuged at 12000g for 5 minutes. The pellet was washed with PBS [pH=7.2] 3 times. The total RNA was extracted by RNA extraction kit (Gene JET RNA Purification Kit Thermo Scientific، Lithuania) and was electrophoresed on 1% agarose gel. cDNA was synthesized by RevertAid First Strand cDNA Synthesis kit (Thermo Scientific, Lithuania) using oligo dT18 primers according to the manufacturer's instructions.

Primers design and PCR amplification of ribosomal protein L3 gene

Primers were designed by making alignment for ribosomal protein L3 gene sequence of Leishmania species by CLC main workbench 7 programs. For *L. major* (Accession NO. XM_001684350.1), *L.donovani* (Accession NO. XM_003862182.1), *L. infantum* (Accession NO. XM_001470124.1) *L.mexicana* (Accession NO. XM_003876912.1). The sequences are identical in the terminal ends of the gene, so we took the mutual 24 bases from the beginning, and 20 bases at the end of the gene sequence. An online application: http://www.idtdna.com/calc/analyzer was used to test the primers. The results demonstrated the melting temperatures and the possibility of conformation of self-dimers, hairpins and heterodimers. According to this application, the melting temperature of forward primer was 66.9ºC and of reverse primer was 66.8 ºC. Restriction enzymes sites had been added to the primers sequence to invent primers that are suitable for amplifying the desired sequence and in the same time make this amplified sequence ready to be recombinant in certain plasmid. To determine which restriction enzymes cut the gene, the sequence of the gene in all species were tested by the application: http://rna.lundberg.gu.se/cutter/. EcoR1 and XbaI restriction enzymes had been chosen. Restriction sites of EcoR1 and XbaI restriction enzymes were added on 5' end of forward and reverse primers respectively. High purified primers were purchased from Alpha DNA, Montreal, Canada. The final sequences for primers were:

Forward primer introduced EcoR1 restriction site; 5' GGA ATT CAT GTC TCA CTG CAA GTT CGA GCA C 3'. Reverse primer: introduced XbaI recognition site; 5' GCT CTA GAT TAC TTC TTC GCG GCC TTT G 3'. EcoR1 and XbaI restriction sites added for direct cloning in the pCI mammalian expression vector are underlined which induces constitutive expression of cloned DNA insert in mammalian cells.

DNA and cDNA of ribosomal protein L3 gene were amplified using Thermal cycler (Bio-Rad, USA) by hot start green master

mix 2x kit (Promega, USA), and the designed primers. PCR protocol was optimized using Gradient annealing temperatures. PCR reactions performed in a 25µl mixture containing: 2.5µl of template DNA (220 ng) or cDNA (240 ng), 12.5µl of PCR master mix 2X, 8.5µl of distilled water and 1µl of each of primers (10 mM). Thermal cycling conditions were 95°C for 5min followed by 35 cycles of 95°C for 1min, 59°C for 45sec and 72°C for 1 min, finally 72°C for 5 min.

Sequence Analysis

Full-length double-stranded sequence analysis was performed on PCR products from cDNA by sequencing, using designed primers mentioned above on automated sequencers [ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit, Perkin-Elmer, Foster City, USA].

Results

Genomic DNA and total RNA extraction

Extracted DNA electrophoresis on a 1% agarose gel showed only one band, (**Figure 1**), which demonstrated the absence of degradation of extracted DNA. The purity of the DNA was 1.82.

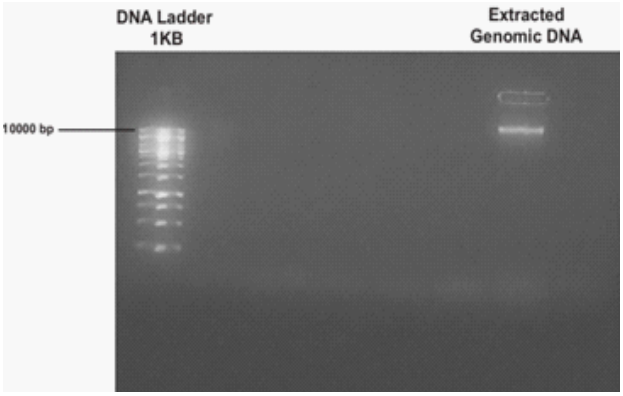

Figure 1: *Electrophoresis of extracted genomic L. tropica DNA on 1% agarose gel stained with ethidium bromide.*

The purity of the extracted RNA was 2.2, that reveals good purity of extracted RNA. The electrophoresis of extracted RNA on a 1.5% agarose gel, (**Figure 2**), showed a standard profile, no DNA bands were noticed so there was no contamination with genomic DNA.

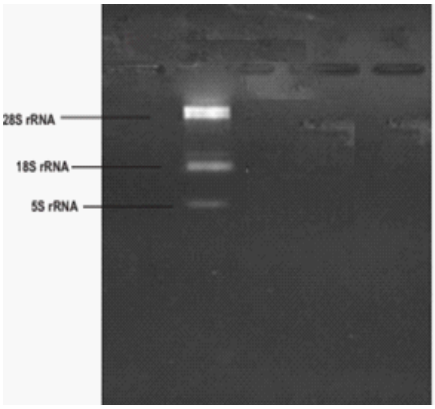

Figure 2: *Electrophoresis of extracted L. tropica RNA on 1.5% agarose gel stained with ethidium bromide.*

Amplification of ribosomal protein L3 gene using both extracted DNA and Synthesized cDNA

PCR protocol was optimised using gradient annealing temperatures, the annealing temperature 59°C was selected due to its high suitability. Gel electrophoresis of ribosomal protein L3 PCR products, on the level of DNA, (**Figure 3**) and cDNA, (**Figure 4**), showed only one band belongs to the gene, and the size of the band, in either case, was approximately 1260 bp which reveals the specificity of used primes.

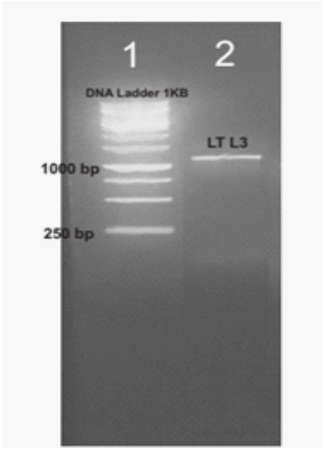

Figure 3: *Amplification products of L. tropica ribosomal protein L3 on 1% agarose gel electrophoresis stained with ethidium bromide, lane 1: DNA ladder 1 kb. Lane 2: L3- DNA amplification product about 1260 bp.*

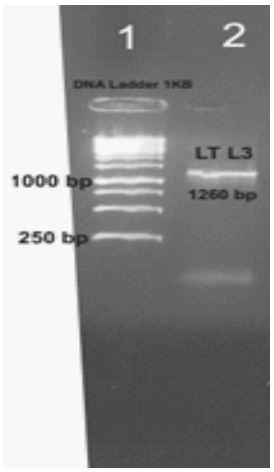

Figure 4: *Amplification products of L. tropica ribosomal protein L3 on 1% agarose gel electrophoresis stained with ethidium bromide. Lane 1: DNA ladder 1 kb, lane 2: L3- cDNA amplification product about 1260 bp.*

Ribosomal protein L3 gene sequencing

The PCR product of ribosomal protein L3 cDNA was specified and sequenced, the sequence has been submitted to the Genbank under the accession number accession NO. MN495878.1 https://www.ncbi.nlm.nih.gov/nuccore/MN495878.1. (Figure 5) shows the complete nucleotide sequence of the L3 1260 pb cDNA. The comparisons of deduced amino acid sequences with other species were made. Proteins contains 419 amino acids were predicted by the deduced amino acid sequences. Geneious Prime programme was used to compare these predicted sequences with the sequences of proteins coded by cDNA of ribosomal protein L3 in other species of Leishmania such as L. major, L. mexicana, L. donovani and L. infantum, an additional file shows this in more detail [see Additional file].

Interestingly, L. tropica L3 gene sequence shows significant similarities with L3 gene of L. infantum [98.81%], L. major [99.44%], L. donovani [94.41%], and L. mexicana [98.33%] using CLC Main Workbench.

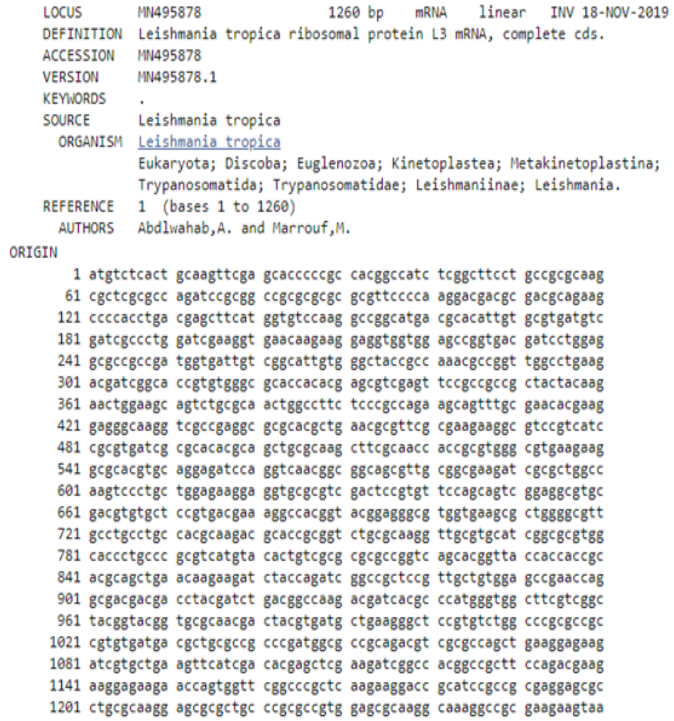

Figure 5: *The Complete Nucleotide Sequence of the L3 cDNA in Syrian Strain of Leishmania tropica.*

Discussion

Leishmaniasis is a widespread and endemic disease in tropical and subtropical areas, reported in 102 countries across the world. Among 0.9 to 1.3 million new cases every year there are about 20 to 30 thousand people could face death. flagellated protozoans of Leishmania parasite species are the causative of the disease that are transmitted by sandfly species. Leishmaniasis involves three original clinical manifesting: Visceral Leishmaniasis (VL) which becomes fatal if remains untreated, Cutaneous Leishmaniasis (CL) which represent the most common form of the disease, and Mucocutaneous Leishmaniasis (ML) [11]. There are a little number of efficient drugs to cutaneous leishmaniasis with the risks of drug resistance to be developed. Nowadays there are no vaccines to prevent leishmaniasis, so, the developing a safe, effective, and affordable vaccine against leishmaniasis infection remains one of the most important public health goals [12]. DNA vaccination strategy represents a promising approach for designing a vaccine against leishmaniasis infection. This method of vaccination capable of elicit the required T-cell immune responses that guarantees the elemintation of intracellular parasite Leishmania [13].

Biochemical studies demonstrate the crucial role of ribosomal protein L3 in the ribosomal catalytic activity [10]. Besides the crucial roles of ribosomal protein L3 in parasite life, many studies illustrate the capacity of ribosomal protein L3 in inducing protection immunity against bacteria [6], parasites [8] leishmania parasite included [9].

In this study we began to explore the presence of ribosomal protein L3 gene in the genome of Leishmania tropica particularly due to the lack of studies about Leishmania tropica genome.

Required primers were designed according to L3 gene sequence in other Leishmania species and the condition of the PCR reaction has been optimized. The optimal annealing temperature for primers was 59°C. Gel electrophoresis of PCR products from both DNA and cDNA showed approximately 1260 bp band that was similar to L3 genes in other Leishmania species. The precise length of L3 gene was given via sequencing and it was 1260 bp and its length was identical with other species. So L3 gene is a part of Leishmania tropica genome and L3 protein is expressed in this parasite also, and the similarity between this gene in Leishmania tropica and other species were more than 95%, these high similarities indicates that infection by different species of Leishmania can controlled by a perfect L3 DNA vaccine.

More than that, the designed primers are specific for L3 gene in Leishmania tropica and gave only one and specific band. This study has chosen ribosomal protein L3 gene to explore its presence in L. tropica genome as first step to invent a DNA vaccine against the infection with L. tropica, which is the main causative agent of majority of cutaneous leishmaniasis cases in Syria. The presence of L3 gene was not demonstrated in L. tropica before, so this present study is the first to detect the presence of L3 gene in Leishmania Syrian strain genome, to prove if there is an expression of the gene in these parasites and to sequence it.

Conclusion

We demonstrated for the first time the presence of ribosomal protein L3 gene in Syrian strain of L. tropica, detect the sequence of the cDNA of ribosomal protein L3 gene and submit this gene to the Genbank with accession number NO. MN495878.1.

Declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Availability of data and materials: All data created or used during this study are openly available from the Leishmania Centre for Epidemiological and Biological Studies, Damascus University.

Competing interests: There are no competing interests.

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Authors' contributions

Alyaa Abdlwahab accomplished all sections of this manuscript as parts of her duties as a master student at Damascus University, Mohammad Maarouf supervised the whole work of the manuscript as AA' supervisor at Damascus university.

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