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Research Article

Investigation the Existence of H2A Gene in *Leishmania Tropica*

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Abstract

Cutaneous Leishmaniasis caused by *Leishmania tropica* is one of the most important health issues in Syria which exacerbated after the war in Syria. Reduction of the high prevalence of Leishmaniasis in Syria requires the availability of an effective vaccine. DNA vaccine has recently provided a promising new approach. Nucleosomal core histones are very important for the parasite's life, their genes showed a protective effect as DNA vaccines against infections by many species of Leishmania. The existence of these genes, like H2A gene, was not proved in *L. tropica*. Our study aimed to investigate the existence of H2A gene in a Syrian strain of *L. tropica* promastigotes and their expression. We isolated DNA and RNA from these parasites, synthesized cDNA, designed the necessary primers for gene`s amplification, and standardized the PCR conditions. This is the first study that proved the existence of this gene in a Syrian strain of L. tropica promastigotes. The sequence of the cDNA H2A gene was defined and published in Gene bank, its size was 399bp. Expression was also proved at the level of cDNA

Keywords: *Leishmania tropica*; H2A gene; Syria

Abbreviations: cDNA: Complementary Deoxy Ribonucleic Acid; DNA: Deoxy Ribonucleic Acid; RNA: Ribonucleic Acid; L.: Leishmania

Introduction

Leishmaniases are infectious diseases caused by several species of the intracellular protozoan of Leishmania, which are transmitted by sandfly [1]. Leishmaniasis has different clinical manifestations, ranging from self-healing cutaneous ulcers to potentially fatal visceral infection [1]. These diseases represent a global public health problem, with an estimated prevalence of 12 million human beings infected. Syria is one of 9 countries have the majority of cutaneous Leishmaniasis cases in the world [2]. Current control is based on chemotherapeutic treatments which are expensive, toxic and associated with high relapse and resistance rates. Vaccination remains the best hope for control of all forms of the disease, so the development of a safe, effective and affordable antileishmanial vaccine is a critical global public-health priority. Immunization with naked DNA is a relatively new approach, which promises to revolutionize the prevention and treatment of infectious diseases [3]. Many studies have been made to find a protective antigen against leishmaniasis and many candidates have been studied for this aim, including histones. Histones are structural proteins found in the nucleus, where they play an important role in the organization and function of chromatin [4]. These highly conserved proteins that elicit strong immune responses are generally designated as panantigens [5]. Specific antibodies for parasite histones, obtained from dogs with visceral leishmaniasis, react against Leishmania H2A [6], H3 [7], H2B and H4 [8] but do not recognize mammalian histones. In the right conditions, these panantigens can provide the immunomodulatory properties needed for vaccine design [9]. So, these histones and their genes are good candidates for vaccine. H2A gene was sequenced in many species of Leshmania, but not in *L. tropica*, and their immunogenicity was tested [10,11]. We chose H2A gene, to design a DNA vaccine against the infection with *Leishmania tropica* which is responsible of the most cutaneous cases in

Syria. The existence of H2A gene, wasn't proved in *L. trpoica*. In this study; A methodical study was first made to know if this gene exists in the genome of a Syrian strain of *Leishmania tropica*, to prove if there is an expression of H2A Gene in these parasites, and to sequence this gene.

Materials and Methods

Primer design for PCR

Sequence of H2A gene in *Leishmania tropica* is not known so we made an alignment for the sequences of this gene in other species using CLC free workbench 7 and Vector NTI Express programs and the clustalw2 and emboss needle tools. Primers were designed using http://www.idtdna.com/calc/analyzer and http://www.geneinfinity.org/sms/sms_primanalysis.html tools. High purified Primers were ordered from VBC- Biotech, Vienna. Final sequences for primers were: (forward, 5'- ATTA GAATTC ATG GCT ACTCCTC-GCAGCG -3'; reverse, 5'- CT TCTAGA CTAAGCGCTCGGTGTCGCCTTG -3'). Underlined are the ECORI and XBAI restriction sites included for direct cloning in the PCI mammalian expression vector (Promega, United States).

DNA extraction

DNA was extracted from the promastigotes of a Syrian strain *Leishmania tropica* (LCEBS, Damascus, Syria). DNA was isolated from Leishmania promastigotes, cultured on RPMI-1640 medium (Lonza, Switzerland) supported with Fetal bovine serum (Cytogen, GmbH, Germany), with DNA extraction kit (Thermo scientific, Lithuania) according to manufacturer's instructions.

RNA isolation and cDNA synthesis

Total RNA was extracted from harvested *Leishmania tropica* promastigotes using TRI reagent (Sigma, St Louis, MO, USA). RNA was quantified by Nano Drop 1000 (Thermo Fisher Scientific MA, USA). RNA-integrity was checked by separating in 1.5% agarose gel. First strand cDNAs were synthesized using Revert Aid First Strand cDNA Synthesis kit (Thermo scientific, Lithuania) according to manufacturer's instructions.

Polymerase chain reaction

DNA and cDNA of H2A gene were amplified by thermal cycler (Bio-Rad, USA) using PCR master mix (Thermo scientific, Lithuania) and the primers mentioned above. PCR protocol was optimized using gradient annealing temperatures. Reactions were performed in 25µl of a mixture containing 1µl of 10µM from each primer, 2, 5µl of template DNA or cDNA (110ng\µl), 12, 5µl of PCR master mix and 8µl of PCR water. Thermal cycling conditions were 95 °C for 5min followed by 35 cycles of 95 °C for 1min, 61.6 °C for 45sec and 72 °C for 1min finally 72 °C for 5 min. PCR products were loaded on a 2% agarose gel, stained with ethidium bromide, and visualized on a UV transilluminator.

Plasmid construction and purification

PCR product containing H2A coding sequence was purified using Gene Jet PCR Purification kit (Thermo scientific, Lithuania) according to manufacturer's instructions. Purified cDNA and the PCI mammalian expression vector (Promega, United States) were double digested with ECORI plus XBAI. Digested cDNA was ligated with the digested PCI mammalian expression vector using t4 DNA ligase (Thermo scientific, Lithuania). PCI-H2A clones were transformed into *E-coli* TOP 10. Recombinant plasmid was extracted from colonies and purified using GF-1 plasmid DNA extraction kit (Vivantis, Malaysia).

Sequences analysis

Full-length double-stranded sequence analysis was performed on purified Recombinant plasmid PCI-H2A by sequencing, using T7 (forward) and PCR 3'(reverse) vector primers on automated sequencers (ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit, Perkin-Elmer, Foster City, USA).

Results and Discussion

Evaluation DNA and RNA quality and purity

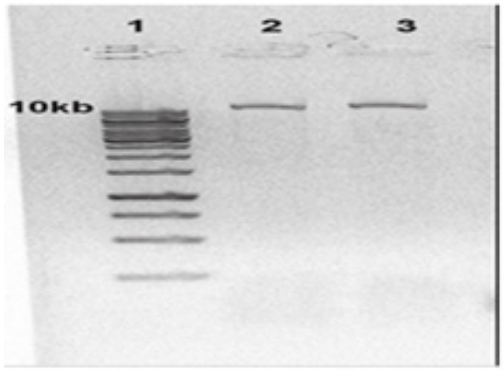

Figure 1: Electrophoresis of extracted *Leishmania tropica* DNA: Lane 1 DNA ladder 1Kb, Lane 2,3 extracted *Leishmania tropica* DNA.

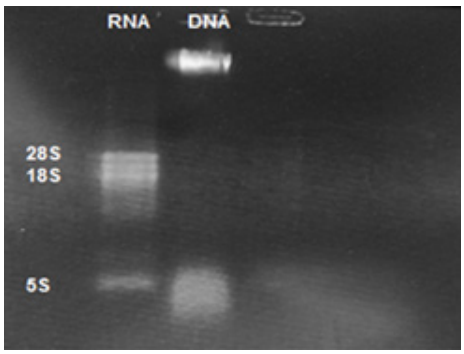

Figure 2: Electrophoresis of extracted *Leishmania tropica* RNA: Lane1 *Leishmania tropica* RNA, Lane 2 extracted *Leishmania tropica* DNA.

Extracted DNA electrophoresis on a 1% agarose gel only showed one band which proves that there isn't any degradation in

the extracted DNA (Figure 1). The purity of the DNA was 1.8 which showed a high degree of purity. Extracted RNA electrophoresis on a 1.5% agarose gel showed a standard profile and there was no contamination with genomic DNA (Figure 2). The purity of the RNA was 2.1 which showed a sufficient degree of purity.

Polymerase chain reaction

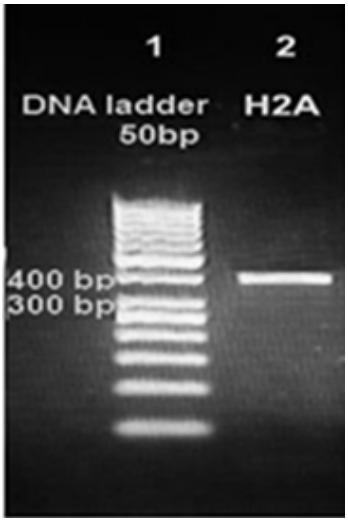

Figure 3: Amplification products of *Leishmania tropica* H2A on 2% agarose gel electrophoresis stained with ethidium bromide. Lane 1 DNA ladder 50bp, lane 2 H2A-DNA amplification product about 400bp.

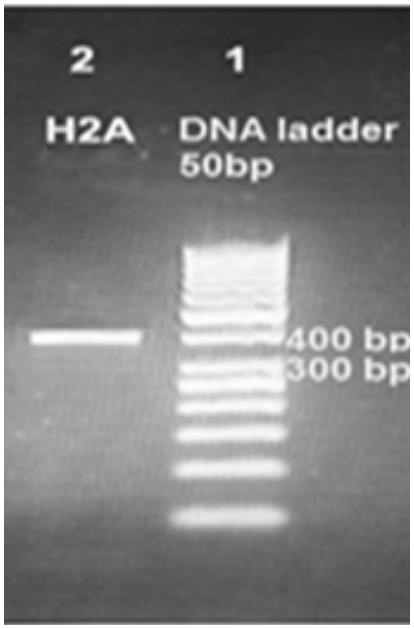

Figure 4: Amplification products of *Leishmania tropica* H2A on 2% agarose gel electrophoresis stained with ethidium bromide. Lane 1 DNA ladder 50bp, lane 2 H2A-cDNA amplification product about 399bp.

PCR protocol was optimized using gradient annealing temperatures and we chose the most suitable annealing temperature which was 61.6 °C. Gel electrophoresis of H2A PCR product on the level of DNA and cDNA showed only one band about 400bp which proves the specificity of our primers. So, we proved the existence of H2A gene in the genome of a Syrian strain of *Leishmania tropica* (Figure 3) and in the cDNA of this strain (Figure 4).

Cloning and sequencing of the H2A cDNA

```
  1 atggctactc ctcgcagcgc caagaaggcc gcccgcaaga gcggctccaa gtccgcgaaa
 61 tgtggtctga tcttcccggt gggccgcgtc ggcgggatga tgcgccgcgg ccagtacgct
121 cgccgcatcg gtgcctctgg cgccgtgtac ctggccgccg tgctggagta cctgacggcg
181 gagctgctgg agctgtccgt gaaggcggcc gcgcagagcg ggaagaagcg gtgccgcctg
241 aacccgcgca ccgtgatgct ggccgcgcgc cacgacgacg acatcggcat gcttctgaag
301 aacgtgacct tgtctcacag cggcgttgtg ccgagcgtca gcaaggcggt ggcaaagaag
361 aagggcggca agaagggcaa ggcgacaccg agcgcgtaa
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Figure 5: Amplification products of *Leishmania tropica* H2A on 2% agarose gel electrophoresis stained with ethidium bromide. Lane 1 DNA ladder 50bp, lane 2 H2A-cDNA amplification product about 399bp.

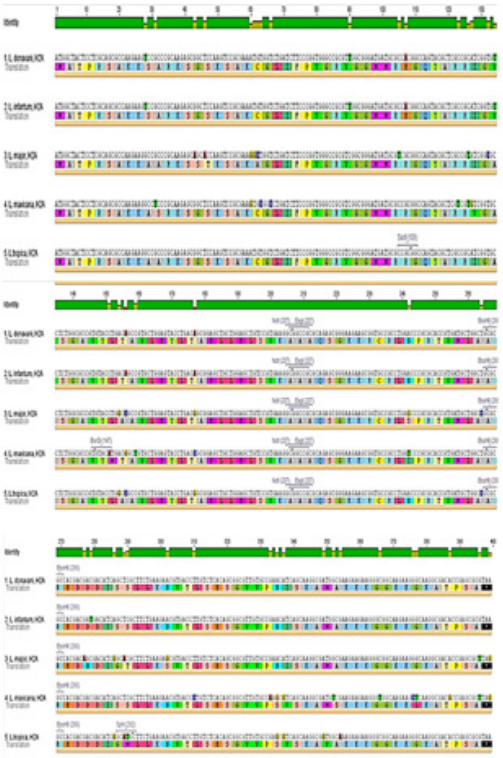

Figure 6: Nucleotide sequences of H2A cDNA for *L.tropica, L.donovani, L.infantum, L.major and L.mexicana.* The deduced amino acid sequence is indicated below the cDNA sequence.

In an effort to clone and characterize the *Leishmania tropica* H2A gene, a cDNA clone was identified and sequenced. The sequence was submitted to the Gene bank under KT956066.1 Accession number. The complete nucleotide sequence of the 399bp cDNA insert, and the deduced amino acid sequence in comparison with other species were showed in (Figures 5) & (Figure 6), respectively. The deduced amino acid sequence predicts a protein containing 132 amino acids. The H2A predicted amino acid sequence was compared with the sequences of proteins coded by cDNA of H2A in

other species of Leishmania such as *L. infantum*, *L. major*, *L. donovania*, *L. mexicana* and *L. braziliensis* using Vector NTI Program. This search revealed that H2A belongs to the histone H2A family. Interestingly, *L. tropica* H2A shows significant similarity with H2A histones of *L. infantum* (92.4%), *L. major* (91.7%), *L. donovania* (92.4%), *L. mexicana* (91.7%) and *L. braziliensis* (78%). The last high similarity shows that a good H2A DNA vaccine could protect from infection by the different species of Leishmania.

Conclusion

Our results proved the existence of H2A gene in the genomic DNA and the cDNA of a Syrian *Leishmania tropica* strain. We proved for the first time the existence of H2A gene in *Leishmania tropica* and defined the sequence of the cDNA of this gene and submitted it to the Gene bank under KT956067 accession number. PCI-H2A clones could now be tested to evaluate their immunogenicity as DNA vaccine against Leishmania species.

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