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Original Article

Preliminary Information of Iranian Lizard *Leishmania* Promastigote Transcriptome Sequencing by Next Generation Sequencing (NGS) Method

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Abstract

Background: A lizard *Leishmania* has been isolated from a lizard (*Agama agilis*) in Iran. Its genome sequence has not been determined, so far.

Methods: The study was done at Shahid Beheshti University of Medical Sciences, Tehran, Iran in 2017-2023. *Leishmania* promastigotes were cultured in $RPMI_{1640}$ culture medium and collected at logarithmic phase by centrigugation. Parasite RNA was extracted by the Qiagene standard kit and its quantity and quality was determined and sequenced by NGS method with Illumina PE machine at BGI Company (China).

Results: The number of 8316 mRNA, 83 tRNA, 63 rRNA, 83 ncRNA, 5 snRNA, 1039 snoRNA, 36 region, and 3 repeat regions, 8343 CDS, 9597 Exon and 9292 Genes were identified in promastigote of Iranian lizard *Leishmania*.

Conclusion: Genomic elements of Iranian lizards *Leishmania* (with unique characteristics) were determined and identified by NGS system.

Introduction

Leishmaniasis is one of the most important skin and visceral infections in Iran. There is no vaccine for field prevention of this infection in the population at risk. The WHO recommended that vaccine research was not useful for field study and that modified traditional methods (such as leishmanization) should be used to control the disease in at-risk populations (1,2). A *Leishmania* was isolated from lizard (*Agama agillis*) in Iran, which is not pathogenic for humans (3). This parasite can be a candidate for leishmanization and because its post-translational modifications is similar to humans, it is useful for the expression of pharmaceutical proteins. For the use for leishmanization as well as the expression of recombinant proteins, there are not much information about its genomic sequence.

The Iranian lizard *Leishmania* has been used for leishmanization (in laboratory animals) and as a host for the expression of several eukaryotic recombinant proteins like Interleukin 12 (4), human

Coagulation factor VII (5), monoclonal antibody against interleukin-2 receptor (6), IL-29 [INF-ɣ] (7) and ameleogenin - the protein used in ossification in dentistry (8).

Ivens et al. identified the genome of *L. major* (9) and Peacock et al. compared the genomes of three species of *Leishmania* (10). The haploid genomes of *Leishmania* species have 32816678 base pairs organized in 36 chromosomes (9) with 911 RNA Genes and 39 pseudogenes were identified (9). Overall, 8272 genes are known to encode proteins. Protein producing *genes* are long polycistronic genes without transcription factors (11, 12). With a new look at *Leishmania*, Myler et al. determined the role of genes in *L. major* (13, 14).

The genetic information of most organisms is in cDNA sequences known as EST (expressed sequence tags) (15). Most *Leishmania* genes do not have introns (16) and PCR product of chromosomal DNA is used for gene cloning (17-21). Gene transcription for protein production in eukaryotes is done by RNA polymerase II, and transcription by RNA polymerase I is for the production of ribosomal RNA. Transcription in kinetoplastids includes RNA polymerase I and trans-splicing mechanism (22).

We aimed to sequence the transcriptome and miRNAome of the Iranian lizard *Leishmania*.

Materials and Methods

Sample collection

This study was done at Shahid Beheshti University of Medical Sciences, Tehran, Iran in 2017-2023. All glass containers were washed with DEPC treated water (water containing diethyl pyrocarbonate) and placed at 250 degrees Celsius for one hour to remove RNase. All required buffers were prepared with DEPC treated water. Glass containers were used for RNA extraction. Iranian lizard *Leishmania* promastigotes were cultured in $RPMI_{1640}$ medium and collected by centrifugation at logarithmic phase Qiagen RNeasy Mini Kit was used for RNA extraction.

Quantitative and qualitative of extracted RNA

The quality, quantity and integrity of total RNA were evaluated using the spectrophotometer Nano Drop1000 and Bioanalyzer 2100 (Agilent Technologies, CA) and using the 28S/18S & 23S/16S test called the RIN test, the samples with the RNA quality score (RIN) > 7.0 were used for RNA sequencing.

Preparation RNA for Sequencing by NGS

The steps were done as protocol in https://www.thermofisher.com/de/de/home/life-science/dna-rna-purification-analysis/rna-extraction/rna-applications/rna-sequencing-sample-preparation.html

Sequencing by Next Generation Sequencing method

This protocol lasts for 3-5 days and includes several steps as follows. Library production and sequencing with Illumina PE device was performed as follows Day 1: RNA isolation, DNase addition and RNA fragmentation. Day 2: cDNA synthesis and integration of dUTP into the second strand. Day 3: preparation of Illumina Paired End sequencing library and dUTP labeled strand destruction. Day 4: Amplification of the cDNA library (Fig. 1).

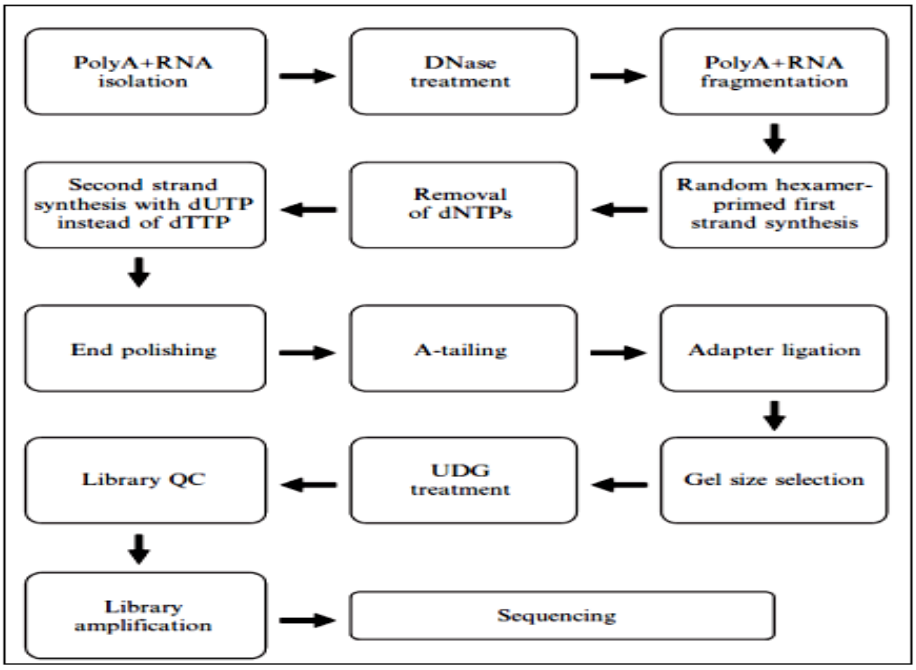

Fig. 1: The steps for sample preparing for NGS

Results

The steps of sample collection and preparation were successfully completed as Fig. 1, and then RNA extraction and purification was performed based on a specific protocol. RNA samples were quantitated and RIN > 7.0 were used for RNA sequencing (Table 1). The RNA samples were sequenced by BGI Company, China, and the raw sequencing data were received and analyzed by experts. This report mentions the number of identified transcripts (Table 2). The results of NGS were classified based on *L. major* strain Friedlin complete genome as reference strain (supplementary data file).

Table 1: Test results of extracted RNA samples

No.	*Sample Number*	*Tube No.*	*Concentration (ng/μl)*	*Volume (μl)*	*Total mass(μg)*	*RIN*	*Test Result*
1	A: 8521803003922	1	36.18	20	0.5789	6.9	Qualified
2	B: 8521803003923	1	51.29	20	0.66	7.2	Qualified

3	C: 8521803003924	1	46	20	0.74	8.1	Qualified

Table 2: Classification of genomic elements identified in the genomic organization of Iranian lizard Leishman by NGS method

Genomic elements	*Number*	*Genomic elements*	*Number*
rRNA	63	mRNA	8316
snoRNA	1039	tRNA	83
snRNA	5	miRNA	83
Sequence Feature	84	Pseudogenes	96
Region	36	CDS	8343
Exon	9597	Repeat region	3
Gene	9292		

Discussion

Leishmania is a protozoan parasite that has similarities and differences compared to other eukaryotic cells. Some of its characteristics, such as polycistronic transcription, are shared with prokaryotic cells (23, 24). The unique characteristics of this parasite have attracted researchers. In recent years, *Leishmania* species have been used as hosts for the production of recombinant proteins (4-8). A suitable host is an essential factor in the production of recombinant proteins**.** Prokaryotes such as *Escherichia coli* grow in inexpensive culture media and have a short multiplication time, which provides high efficiency in the production of recombinant proteins. However, prokaryotes do not perform post-translational modifications such as glycosylation, phosphorylation, and carboxylation. Some eukaryotic proteins are non-functional after translation in *E. coli*, and some of them accumulate as inclusion bodies in the cytoplasm of the host cell. If these proteins are produced in a prokaryotic host, they will not fold properly, and their third and fourth structure are not formed (25.). Proliferation of yeasts such as *Pichia pastoris*, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* also require long culture time and post-translational modifications in these microorganisms are not complete processes (26). Culture media for eukaryotic cells are expensive and require specialized culture conditions and laboratory equipment. Because *Leishmania* is easily and inexpensively maintained in NNN culture medium and can multiply rapidly; it is preferred over others. This parasite can also perform post-translational modifications (22,27). These cause *Leishmania* species to be considered as a suitable host for the production of recombinant pharmaceutical proteins (27-30). Attempts have been made to produce some therapeutic proteins in this parasite (31-34). However, more research is needed before they can be widely used as hosts for the production of recombinant proteins. Researchers in biochemistry, pharmacology and immunology, who are involved in the development of new drugs as well as the production and testing of vaccines, need appropriate cell models. *Leishmania* species are expected to be suitable candidates for these cases (35,36).

If *Leishmania* is used for protein expression, we need some information on its proteases, as may lysis recombinant protein. In this research, the transcriptome (mRNA and ncRNA) of Iranian lizard *Leishmania* was investigated and comprehensive and complete information was obtained about this native and non-pathogenic *Leishmania*. Considering the post-translational changes of this parasite, which is similar to humans, it is a good suggestion for use in the production of recombinant proteins. The *Leishmania* expression system is almost identi-

cal to the RBS method in prokaryotes, while the post-translational modifications of the protein produced through this system are almost identical to those of mammalian proteins. Multisubunit proteins have the unique three-dimensional structures of eukaryotic cells and are similar to mammalian proteins in terms of post-translational modifications (37). The production of multi-subunit proteins in *Leishmania* is done at a cheaper price and does not require extensive biological safety facilities, compared to mammalian cells, it has a high growth rate, its culture medium is cheap, and it grows at a temperature of 26 degrees Celsius, and does not require Incubator does not have CO2 (37-38).

Conclusion

For the first time, genomic elements of Iranian lizards *Leishmania* (with unique characteristics) were determined and identified by NGS system.

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Conflict of interest

The authors declare that there is no conflict of interest.

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