PLOS | ONE

RESEARCH ARTICLE

Molecular and Functional Characterization of ssDNA Aptamers that Specifically Bind *Leishmania infantum* PABP

Natalia Guerra-Pérez[1¤a], Edurne Ramos[1¤b], Marta García-Hernández[2], Celia Pinto[1], Manuel Soto[3], M. Elena Martín[1‡], Víctor M. González[1‡*]

1 Laboratory of aptamers, Servicio de Bioquímica-Investigación, IRYCIS-Hospital Ramón y Cajal, Madrid, Spain, **2** Aptus Biotech SL, Parque Científico de Madrid, Campus de Cantoblanco, Madrid, Spain, **3** Departamento de Biología Molecular, Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Universidad Autónoma de Madrid, Madrid, Spain

¤a Current address: Center for Biomedical Research, Population Council, New York, New York, United States of America
¤b Current address: Departamento de Patología, IRYCIS-Hospital Ramón y Cajal, Madrid, Spain
‡ These authors are joint senior authors on this work.
* victor.m.gonzalez@hrc.es

OPEN ACCESS

Citation: Guerra-Pérez N, Ramos E, García-Hernández M, Pinto C, Soto M, Martín ME, et al. (2015) Molecular and Functional Characterization of ssDNA Aptamers that Specifically Bind *Leishmania infantum* PABP. PLoS ONE 10(10): e0140048. doi:10.1371/journal.pone.0140048

Editor: Henk D. F. H. Schallig, Royal Tropical Institute, NETHERLANDS

Received: April 20, 2015

Accepted: September 21, 2015

Published: October 12, 2015

Data Availability Statement: All relevant data are within the paper.

Funding: This work was supported by Grants PI05/0453 and SAF2010-21663 from the Ministerio de Economia y Competitividad (Spain). MEM and VMG are researchers from FIBio-HRC. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. Marta Garcia-Hernández is employed by Aptus Biotech SL. The funder provided support in the form of salaries for MG-H, but did not have any additional role in the study design, data collection and analysis,

Abstract

Summary

A poly (A)-binding protein from *Leishmania infantum* (LiPABP) has been recently cloned and characterized in our laboratory. Although this protein shows a very high homology with PABPs from other eukaryotic organisms including mammals and other parasites, exist divergences along the sequence that convert them in potential diagnostic markers and/or therapeutics targets. Aptamers are oligonucleotide ligands that are selected *in vitro* by their affinity and specificity for the target as a consequence of the particular tertiary structure that they are able to acquire depending on their sequence. Development of high-affinity molecules with the ability to recognize specifically *Leishmania* proteins is essential for the progress of this kind of study.

Results

We have selected a ssDNA aptamer population against a recombinant 6xHIS–LiPABP protein (rLiPABP) that is able to recognize the target with a low Kd. Cloning, sequencing and *in silico* analysis of the aptamers obtained from the population yielded three aptamers (ApPABP#3, ApPABP#7 and ApPABP#11) that significantly bound to PABP with higher affinity than the naïve population. These aptamers were analyzed by ELONA and slot blot to establish affinity and specificity for rLiPABP. Results demonstrated that the three aptamers have high affinity and specificity for the target and that they are able to detect an endogenous LiPABP (eLiPABP) protein amount corresponding to 2500 *L*. *infantum* promastigotes in a significant manner. The functional analysis of the aptamers also revealed that ApPABP#11 disrupts the binding of both Myc-LiPABP and eLiPABP to poly (A) *in vitro*. On the other hand, these aptamers are able to bind and purify LiPABP from complex mixes.

42. Barfod A, Persson T, Lindh J. In vitro selection of RNA aptamers against a conserved region of the Plasmodium falciparum erythrocyte membrane protein 1. Parasitology Research. 2009; 105(6):1557–66. doi: 10.1007/s00436-009-1583-x PMID: WOS:000270977800009.

43. Ulrich H, Magdesian MH, Alves MJM, Colli W. In vitro selection of RNA aptamers that bind to cell adhesion receptors of Trypanosoma cruzi and inhibit cell invasion. Journal of Biological Chemistry. 2002; 277(23):20756–62. doi: 10.1074/jbc.M111859200 PMID: WOS:000176204500085.

44. Niles JC, DeRisi JL, Marletta MA. Inhibiting Plasmodium falciparum growth and heme detoxification pathway using heme-binding DNA aptamers. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106(32):13266–71. doi: 10.1073/pnas.0906370106 PMID: WOS:000268877300024.

45. Bhattacharyya SN, Chatterjee S, Adhya S. Mitochondrial RNA import in Leishmania tropica: aptamers homologous to multiple tRNA domains that interact cooperatively or antagonistically at the inner membrane. Mol Cell Biol. 2002; 22(12):4372–82. Epub 2002/05/25. PMID: 12024047.

46. Homann M, Goringer HU. Uptake and intracellular transport of RNA aptamers in African trypanosomes suggest therapeutic "piggy-back" approach. Bioorganic & Medicinal Chemistry. 2001; 9(10):2571–80. PMID: 11557345. doi: 10.1038/srep14579

47. Lorger M, Engstler M, Homann M, Goringer HU. Targeting the variable surface of African trypanosomes with variant surface glycoprotein-specific, serum-stable RNA aptamers. Eukaryotic Cell. 2003; 2(1):84–94. doi: 10.1128/ec.2.1.84–94.2003 PMID: WOS:000180988400009.

48. Bhattacharyya SN, Chatterjee S, Adhya S. Mitochondrial RNA Import in Leishmania tropica: Aptamers Homologous to Multiple tRNA Domains That Interact Cooperatively or Antagonistically at the Inner Membrane. Mol Cell Biol. 2002; 22(12):4372–82. doi: 10.1128/mcb.22.12.4372–4382.2002 PMID: 12024047

49. Kolesnikova O, Kazakova H, Comte C, Steinberg S, Kamenski P, Martin RP, et al. Selection of RNA aptamers imported into yeast and human mitochondria. RNA. 2010; 16(5):926–41. doi: 10.1261/rna.1914110 PMID: 20348443