Institut Pasteur
Microbes and Infection 18 (2016) 369–372
www.elsevier.com/locate/micinf

Short communication

Suppression of host PTEN gene expression for *Leishmania donovani* survival in Indian visceral leishmaniasis

Medhavi Sudarshan, Toolika Singh, Bhawana Singh, Jaya Chakravarty, Shyam Sundar*

Department of Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221 005, UP, India

Received 18 June 2015; accepted 31 December 2015
Available online 14 January 2016

Abstract

Lipid phosphatase, PTEN is amongst the host gene actively involved in determining disease susceptibility. Expression of *pten* and other genes in vicinity *egr1* & *4e-bp1* were evaluated in splenic tissue before and after treatment in visceral leishmaniasis patients. Lower expression of *egr1* in correlation with *pten* suppressed *4e-bp1* gene in active cases. The higher levels of *pten* mRNA expression post treatment confirmed its role in effective clearance of *Leishmania*. Therefore, it is hypothesized that lower mRNA expression of *pten* is due to suppression of *egr1* activates PI3K signaling bestowing host the ability to cope up infection and continue its normal metabolic machinery.

Keywords: Visceral leishmaniasis; *pten*; *egr*1; *4e-bp1*; mRNA expression; PI3K signaling

1. Introduction

Leishmania donovani, the causative agent of visceral leishmaniasis in Indian population, is an obligate intracellular parasite which infects macrophages and neutrophils. The interplay between the parasite and its host is a complex process, in which the paramount interest of the parasite is to restrict the immune and microbicidal activities of the host macrophages, while keeping it alive as a nutritional source [1]. Global gene expression analysis in murine macrophage has suggested that infection by *Leishmania* causes general suppression of gene expression [2], but the story in human macrophage remains to be completely different where genes behave differently. During acute infection, spleen is believed to participate in the clearance of parasite from the circulation as well as providing a strong hematopoietic response. For effective defense against *Leishmania* infection negative regulation of PI3K pathway is essential [3].

In general, *Leishmania* alters the functionality of macrophage by suppressing several cellular functions, such as gene expression and phosphorylation. *Leishmania* secretes a factor (or factors) into the cytosol of infected cells that brings about activation of the PI3K directly or indirectly, leading to changes in cell regulation thereby favoring the establishment of infection. When talking about leishmanial infection there are studies from murine model that suggests for the negative regulation of PI3K pathway as an important factor for effective defense [3]. Among different phosphorylated derivatives of the lipid, phosphotidylinositol is a preferential substrate for PTEN, which plays diverse role in cellular signaling. The PTEN, tumor suppressor protein is a phosphoinositide 3-phosphatase with only limited potential to dephosphorylate protein substrates [4]. It metabolizes phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P3), acting in direct antagonism to growth factor and stimulates PI3-kinases [5]. PTEN specifically dephosphorylates the 3-position on PtdIns, predominantly PtdIns(3,4,5)P3, to generate PtdIns(4,5)P2 [6]. By limiting the amount of PIP_3

* Corresponding author. Infectious Disease Research Laboratory, Department of Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, India. Tel.: +91 542 2369 632; fax: +91 542 2367 568.
E-mail address: drshyamsundar@hotmail.com (S. Sundar).

http://dx.doi.org/10.1016/j.micinf.2015.12.008
1286-4579/

available within the cell, PTEN directly opposes PI3K activity and influences the selection of developing thymocytes as well as the activation requirement of mature T-cells [7]. Further PTEN deficient cells have high level of 4E-BP1 phosphorylation [8]. 4E-BP1 has been known for its role in protein synthesis within the macrophage [9]. Signaling pathway PI3K/AkT/mTOR controls 4E-BP1 phosphorylation while little is known about the regulation of its expression. Expression of 4E-BP1 is controlled primarily at a transcription level, the data from deletion studies of *4e-bp*1/2 suggested for the reduced parasite load in macrophage *ex vivo* and decreased susceptibility to cutaneous leishmaniasis in *vivo* [10]. The promastigote form of *Leishmania* (using its GP63 activity) promotes its survival through downregulation of macrophage protein synthesis. Macrophage lacking pten has reduced ability to eliminate *L.major* infection and Egr1 transcription factor directly activates *pten* transcription [3,11].

Therefore, in light of the known role of PTEN and the recent genetic evidence for its involvement in host responses to *Leishmania* infection, the aim of this study was to look at *pten* as well as its upstream and downstream gene expression at the RNA level at different time interval, i.e. before and after treatment of human visceral leishmaniasis to know its involvement in disease cure.

2. Material and method

Since the spleen is a major focus for parasite growth inside macrophages in VL, splenic biopsies were taken as part of routine diagnostic procedure at the Kala Azar Medical Research Centre, Muzaffarpur, Bihar State, India.

Pre and post treated patient's splenic samples were collected in 5xRNA Later (AMBION Inc., Austin, Texas, USA) during 2010–2012, transported to Varanasi at 4 °C and stored at −80 °C until RNA was isolated. The details regarding age and sex, splenic parasites and drug administered were recorded for each patient. Consent form was taken from patients considering ethical issues. Total RNA was isolated using RNeasy tissue kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. Sample quality and integrity was assessed by ND-2000 spectrophotometer (Thermo Fischer Scientific Wilmington, DE, USA) and agarose (Sigma Aldrich Chemicals, St Louis, MO, USA) gel electrophoresis. 500 ng of RNA was reverse transcribed using the High Capacity cDNA synthesis kit (Applied Biosystems, Foster City, CA, USA). SYBR Green based gene expression assay was perforemed on genes *pten, egr1* and *4e-bp1* while TaqMan based assay were used for analyzing IL-10 and IFN-γ; primer sequences shown in Table 1. Comparative delta Ct was performed using GAPDH as endogenous control on 7500 REAL TIME PCR platform (ABI, Foster City CA, USA). Experiment was performed on 16 paired pre- and post-treatment as day-0 and day-dis splenic aspirates from VL patients with appropriate no RT and no template controls included in each plate. All samples were run in duplicate.

Table 1
(A) Primer sequences used for SYBR green based gene expression assay (Integrated DNA technology), (B) FAM-MGB labeled primer/probe for IL-10 and IFN-γ (Applied Bio system).

A

Primer	Sequence
Egr 1	For 5′ CGCTTCTCAGTGTTCCCCGC 3′
	Rev 5′ CAGGCTGGAGAGCTGGTGTCG 3′
Pten	For 5′ CGAACTGGTGTAATGATATGT 3′
	Rev 5′ CATGAACTTGTCTTCCCGT 3′
4e-bp1	For 5′ GCAGGAGCTGCCACCCCAAG 3′
	Rev 5′ GCAGCGAGGGGCTGGAACTG 3′
Gapdh	For AATGAAGGGGTCATTCATCG
	Rev AAGGTGAAGGTCGGAGTCAA

B

Gene	TaqMan assay
IL-10	Hs00961622_m1
IFN-Y	Hs01066118_m1
18SrRNA	4333760F

IFN-Y and IL-10 expression of patients were also studied for VL patients before and after treatment.

Results were analyzed by 7500 software v.2.0.1 and Graph pad prism 5 (version 5.00 for Windows, Graph Pad Software, San Diego California USA, www.graphpad.com). The significance of differences between Day-0 and D-dis (D-30) treated groups were determined using paired Student's t-test.

3. Result

Paired Student's t-tests showed significant differences in expression of *pten*, *egr1* and *4e-bp1* at Day-0 and Day-Discharge state. There was less expression of these genes at active stage of *L. donovani* infection compared to treated state with p value of less than 0.05 (Fig. 1). There is more expression of IL-10 and IFN-Y in active disease state compare to discharge condition of VL patients (Fig. 2.)

4. Discussion

PTEN is negative regulator of PI3K pathway [12], in this study we showed reduction of *pten* mRNA expression in active VL. The transcription of *pten* is directly controlled by Egr1 transcription factor [11]. mRNA expression of *egr1* was also studied and found to be less in active disease condition. Therefore, it can be assumed that reduced expression of *egr1* caused lower pten levels and thus, in turn might be involved in activation of PI3K signaling. In addition to the inactivation of 4E-BP1 *via*. hyperphosphorylation, signaling through PI3K pathway silences *4e-bp1* expression [13]. In our study *4e-bp1* gene expression was also suppressed. The study of the transcriptional response of macrophage to *Leishmania* infection may explain the survival and replication of parasite in the macrophage hostile environment which is lethal to other microbes. *In vitro*, PTEN deficient (knockout) macrophages showed a reduced ability to kill parasite in response to IFN-γ treatment [3]. Loss of PTEN enhances T helper cells function.