

The miR-125a-3p is Associated With Leprosy Phenotypes and Correlated With Bacillary Index

Nadja de Lima Santana

Serviço de Imunologia da Universidade Federal da Bahia

Tainã Souza do Lago

Serviço de Imunologia da Universidade Federal da Bahia

Thaillamar Silva Vieira

Serviço de Imunologia da Universidade Federal da Bahia

Thyago Leal-Calvo

Fundação Oswaldo Cruz-FIOCRUZ

Paulo Roberto Lima Machado

Instituto Nacional de Ciência e Tecnologia em Doenças Tropicais

Léa Cristina Castellucci (✉ leacastel@hotmail.com)

Instituto Nacional de Ciência e Tecnologia em Doenças Tropicais

Research Article

Keywords: Leprosy, miR-125, Phenotypes, Bacillary Index

Posted Date: August 5th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-763395/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background. *Mycobacterium leprae* infects skin and peripheral nerves causing a broad of clinical forms. Data have shown that the miR-125a-3p can influence immune mechanisms such as autophagy as well as to target genes leading to abnormal proliferation, metastasis, and invasion of cells.

Methods. Here we used quantitative real time PCR (qPCR) to evaluate the miR-125a-3p expression pattern as a marker for leprosy phenotypes in biopsies obtained from patients with and without reactions. Data were analysed according to clinical forms and bacillary index (BI).

Results. Our results show a significant increase in the miR-125a-3p expression in paucibacillary (PB) vs multibacillary (MB) ($p = 0.007$) and vs RR (reversal reactions) ($p = 0.005$), respectively; and also a higher expression in patients with erythema nodosum leprosum (ENL) vs MB without reactions ($p = 0.002$). In addition, there was a positive correlation between the miR-125a-3p expression and BI in patients with reactions ($r=0,81$; $p=0,002$).

Conclusions. All together we underpin a role for miR-125-3p in leprosy pathogenesis, raising the hypothesis that this miR might have a distinct role in PB and ENL forms, influencing mechanisms such as apoptosis and autophagy according to the local context. A functional study should help to validate the miR-125a-3p as a potential therapeutic target for leprosy treatment.

Background:

Leprosy is a spectral disease, comprising a variety of clinical presentations ranging from the tuberculoid to lepromatous poles, which correspond respectively to the paucibacillary and multibacillary forms of the WHO classification [1]. In 1966, Ridley and Jopling created a classification that comprise the wide different clinical manifestations: indeterminate, tuberculoid, borderlines or lepromatous. Additionally, circa of 30-40% of patients develops sudden and acute inflammatory episodes as so-called reactions. Type 1 or RR present clinically with neuritis or erythema or edema of the skin lesions; type 2 or ENL, involves the appearance of subcutaneous nodules and symptoms such as fever, arthritis, neuritis, among others [2]. MicroRNAs are well-known small noncoding RNA molecules that control gene expression by interacting with mRNAs in practically all biological processes. By base-pairing to mRNAs, miRNAs can inhibit the translation or increase mRNA turnover [3]. Previous studies have shown that the expression levels of both miR-125a-3p/5p can alter host macrophages profiles [4] and that the miR-125a-3p can inhibit host defenses against mycobacterial infection by targeting the gene encoding autophagy UV radiation-resistance-associated protein [5]. Together, these data suggest that miR-125a may inhibit innate macrophage responses by regulating macrophage differentiation, inflammation, and autophagy [6]. Here we investigate whether miR-125a-3p are associated with leprosy phenotypes through its expression in skin biopsies taken from patients with and without reactions.

Methods:

Twenty-eight skin biopsies from leprosy patients (7 PB, 7 MB, 6 RR and 8 ENL), both sex, age mean 45.89 ± 16.30, were collected before treatment at the outpatients leprosy clinics at the Hospital Universitário Edgard Santos and the Couto Maia Institute. Both are considered reference centers for treatment of the disease with patients diagnosed according to the Brazilian's Ministry of Health guidelines. Written informed consent was obtained from all patients after approval of the study by the Ethics Committee from the Federal University of Bahia (CEP 2.149.677), attending the recommendations of Resolution 466/12 of the National Health Council for research with human beings and the Declaration of Helsinki. Tissue RNA was obtained by the TRIzol® RNA isolation method. Gene expression of miR-125a-3p was performed using a pre-designed assay (miRCURY LNA SYBR, QIAGEN) following manufacturer's instructions and using the 7500 Real Time PCR System device (Applied Biosystems®).

Results:

The U6snRNA, miR-146a-5p and miR-511-3p normalizers were identified using the RefFinder program (<https://www.heartcure.com.au/reffinder/>) to validate the qPCR. Statistical data regarding biopsies were generated by Welch's t test and for the analysis of bacillary index the Spearman's Correlation test, using GraphPad Prism8. Our analysis showed a higher expression of miR-125a-3p in the PB group as compared to MB (p = 0.007) and RR groups (p = 0.005), respectively; and also, a higher expression in the group of patients with ENL as compared to MB without reactions (p = 0.002). However, there was no difference by comparing all patients without reactions (PB + MB) *versus* patients with reactions (RR + ENL) as shown in Table 1, (Figure 1). Additionally, we also observed a positive correlation between the miR-125a-3p expression and bacillary index (BI) in patients with reactions (Figure 2).

Discussion:

Members of Bcl-2 family and others involved in apoptosis are an important group of miR-125 targets [7]. In lesions of LL patients, a negative regulation of all miRNAs that act directly on the anti-apoptotic genes such as *BCL2* and *MCL1* was observed [8]. Consistent with this, in another study with untreated patients, a low detection of apoptosis and abundant expression of *BCL2* was observed in lepromatous (LL) samples where both *BCL2* and *MCL1* genes were induced by *M. leprae* in monocytes [9,10]. These data suggest that in LL patients there is a negative regulation of apoptosis favoring the persistence and multiplication of bacilli in macrophages as one of the mechanisms in favour of *M. leprae* [9]. In addition, the miR-125 family also targets *ERBB2* [11], a gene that promotes the binding of *M. leprae* to myelinated Schwann cells through the Erk1/2 signaling pathway, which results in Schwann cell demyelination and creates an environment that contributes *M. leprae* proliferation, leading to nerve damage [12]. Taken together, it can be thought that the greater expression of miR-125a-3p in PB biopsies that we observed may be a factor that contributes to a more efficient apoptotic response characteristic of this clinical form, contributing to a milder disease phenotype. Interestingly, miR-125a-3p was also more expressed in individuals with ENL, when compared to MB individuals without reactions. Similarly, Cleverson et al., 2017, identified a differential expression of miR-125b-2, in the type 2 reactions [13]. However, the mechanism by which miR-

125 participates in the initiation and / or maintenance of the type 2 reaction, in leprosy it is not known. In a model using *M.tuberculosis* (Mtb), it has been shown that increased expression of miR-125a-3p inhibits the activation of autophagy and antimicrobial effects against Mtb via TLR2 and MyD88 in macrophages [14]. The recruitment of MyD88 or TRIF adapter molecules by TLR after ligand engagement is crucial for the activation of a variety of inflammatory mediators in macrophages after mycobacterial infection [15]. In another study associated with human cancers, it was observed that miR-125a increases the invasive potential in urothelial carcinomas [16]. Data such as these are relevant, as they call attention to mechanisms linked to processes of dissemination and metastasis that could justify the action of this miR in ENL, where the disseminated deposits of *M. leprae* antigens resulting in a systemic disease. Coherent with this, we also observed that in patients with RR and ENL there was a positive correlation between the IB and the expression of miR-125a-3p. ENL is a deployment of leprosy that has a distinct immune response in relation to other clinical forms. The role of miR-125a-3p in this case might be unlike PB considering that different triggers could be driving the scene. Therefore, there are two possible explanations: a) The increase in miR-125a-3p expression in ENL would inhibit mechanisms of autophagy, promoting metastasis that in turn mirrors the correlation with a high bacillary index; b) Alternatively, this correlation would reflect an increase in miR-125a-3p as an “attempt” to regulate apoptosis and contain the spread of the bacillus. Members of the miR-125 family have been validated to act either as disease-suppressing or disease-promoting functions in certain contexts such as tumor metastasis and infections. It seems that the role played by this miRNA in PB and ENL are in fact distinct. The information provided in this article opens the perspective for a functional study that might help elucidate mechanisms to validate the miR-125a-3p as a possible therapeutic target for leprosy treatment.

Conclusion:

This work underpins the role of miRNAs with immunological and clinical parameters of leprosy, reinforcing that epigenetic mechanisms underlying the disease regulates its pathogenesis. Functional studies will be important to validate the findings described here.

Declarations

Ethics approval and consent to participate:

The study has been approved by the Ethical Committee of the Hospital Universitário Prof. Edgar Santos, Federal University of Bahia, number 2.149.677. All patients read and signed an informed consent form.

Consent for publication:

Not applicable.

Availability of data and material:

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests:

The authors declare that they have no competing interests.

Funding:

National Institute of Science and Technology in Tropical Diseases, Brazil (Nº 573839/2008-5) and CAPES by granting scholarships.

Authors' contributions:

PRM diagnosed, treated and included the patients in the study. NLS and TSV collected the samples and extracted the RNA from the patients. NLS and TSL performed the gene expression experiments. NLS and TL-C analyzed the data. LCC supervised the samples processing and wrote the manuscript. Supervised the data and manuscript preparation. All authors read and approved the final manuscript.

Acknowledgements:

We thank the staff of outpatients leprosy clinics from Hospital Universitário Professor Edgard Santos and the Couto Maia Institute for the logistical support in the sample collection, Dr. Rosângela Cunha for the clinical care and Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB) for supporting this research. We also thank the Fundação Maria Emília Freire de Carvalho for aid this manuscript publication.

References

1. Mira MT, *Genetic host resistance and susceptibility to leprosy. Microbes and Infection.* 2006; v. **8**, n. 4, p. 1124-1131. doi: 10.1016/j.micinf.2005.10.024.
2. Ribeiro SLE, et al, *Vasculite na hanseníase mimetizando doenças reumáticas.* Revista Brasileira de Reumatologia. 2007; v. **47**, p. 140-144. doi: 10.1590/s0482-50042007000200013.
3. Filipowicz W, Bhattacharyya SN, Sonenberg N., *Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight?* Nat Rev Genet. 2008; **9** 102–114. doi:10.1038/nrg2290.
4. Schnitger AK, Machova A, Mueller RU, et al., *Listeria monocytogenes infection in macrophages induces vacuolar-dependent host miRNA response.* PLoS One. 2011; **6**:e27435. doi: 10.1371/journal.pone.0027435.
5. Kim JK, Yuk JM, Kim SY, et al., *MicroRNA-125a Inhibits Autophagy Activation and Antimicrobial Responses during Mycobacterial Infection.* J Immunol. 2015;**194**:5355–5365. doi: 10.4049/jimmunol.1402557.

6. Lee Hye-Mi; Kim TS, Jo, Eun-Kyeong, *MiR-146 and miR-125 in the regulation of innate immunity and inflammation*. BMB Reports. 2016; **49**(6), 311–318. doi:10.5483/BMBRep.2016.49.6.056.
7. Sun YM, Lin KY, Chen YQ., *Diverse functions of miR-125 family in different cell contexts*. Journal of Hematology & Oncology. Review. 2013;**6**:6. doi: 10.1186/1756-8722-6-6.
8. Salgado CG, Pinto P, Bouth RC, Gobbo AR, Messias ACC, Sandoval TV, Santos AMR, Moreira FC, Vidal AF, Goulart LR, Barreto JG, Silva MB, Frade MAC, Spencer JS, Santos S and Ribeiro-dos-Santos Â., *miRNome Expression Analysis Reveals New Players on Leprosy Immune Physiopathology*. Frontiers in Immunology. 2018; **9**, 463-. doi:10.3389/fimmu.2018.00463.
9. De Souza VNB, Nogueira MES, Belone ADE FF, Soares CT., *Analysis of apoptosis and Bcl-2 expression in polar forms of leprosy*. FEMS Immunol Med Microbiol. 2010; **60**:270–4. doi:10.1111/j.1574-695X. 2010.00746.x.
10. Hasan Z, Ashraf M, Tayyebi A, Hussain R. M., *Lepae inhibits apoptosis in THP-1 cells by downregulation of Bad and Bak and upregulation of Mcl-1 gene expression*. BMC Microbiol. 2006; **6**:78. doi:10.1186/1471-2180-6-78.
11. Scott GK, Goga A, Bhaumik D, Berger CE, Sullivan CS, Benz CC., *Coordinate Suppression of ERBB2 and ERBB3 by Enforced Expression of Micro-RNA miR-125a or miR-125b*. J of Biol Chem. 2007; **282**(2), 1479–1486. doi:10.1074/jbc.M609383200.
12. Tapinos N, Ohnishi M, Rambukkana A., *ErbB2 receptor tyrosine kinase signaling mediates early demyelination induced by leprosy bacilli*. Nature Medicine.2006; **12** (8): 961-966. doi:10.1038/nm1433.
13. Soares, Cleverson T, Trombone, Ana PF, Fachin, Luciana RV, Rosa, Patricia S, Ghidella, Cássio C, Ramalho, Rodrigo F, Pinilla, Mabel G, Carvalho, Alex F, Carrara, Dirce N, Soares, Fernando A, Belone, Andrea FF., *Differential Expression of MicroRNAs in Leprosy Skin Lesions*. Frontiers in Immunology. 2017; **8**(), 1035–. doi:10.3389/fimmu.2017.0103.
14. Kim JK, Yuk JM, Kim SY, Kim TS, Jin HS, Yang CS, Jo EK., *MicroRNA 125a inhibits autophagy activation and antimicrobial responses during mycobacterial infection*. J. Immunol. **194** (2015) 5355–5365.
15. Kleinnijenhuis J, Oosting M, Joosten, Leo AB, Netea MG, Crevel RV., *Innate immune recognition of Mycobacterium tuberculosis*. Clin. Dev. Immunol. 2011; 2011:405310. doi: 10.1155/2011/405310.
16. Ratert N, Meyer H-A, Jung M, Mollenkopf H-J, Wagner I, Miller K, Kilic E, Erbersdobler A, Weikert S, Jung K., *Reference miRNAs for miRNAome analysis of urothelial carcinomas*. PLoS One. 2012, **7**(6): e39309. doi:10.1371/journal.pone.0039309.

Tables

TABLE 1

miR-125a-3p differential expression in leprosy skin lesions

Comparison groups	log2FC	mean 1	mean 2	P-value*	95%CI lower	95%CI upper
PB vs MB	-1.59	-5.38	-6.98	0.0078	-2.67	-0.51
PB vs RR	1.66	-5.38	-7.04	0.005	0.63	2.69
ENL vs MB	1.73	-5.25	-6.98	0.0028	0.75	2.71
PB+MB vs RR+ENL	-0.1236	-6.11831	-5.9947	0.79324	-1.08796	0.840756

PB - paucibacillary; MB – multibacillary; ENL – erythema nodosum leprosum; RR- reversal reactions. Values expressed in logfc, analyzed by Welch's t test.

Figures

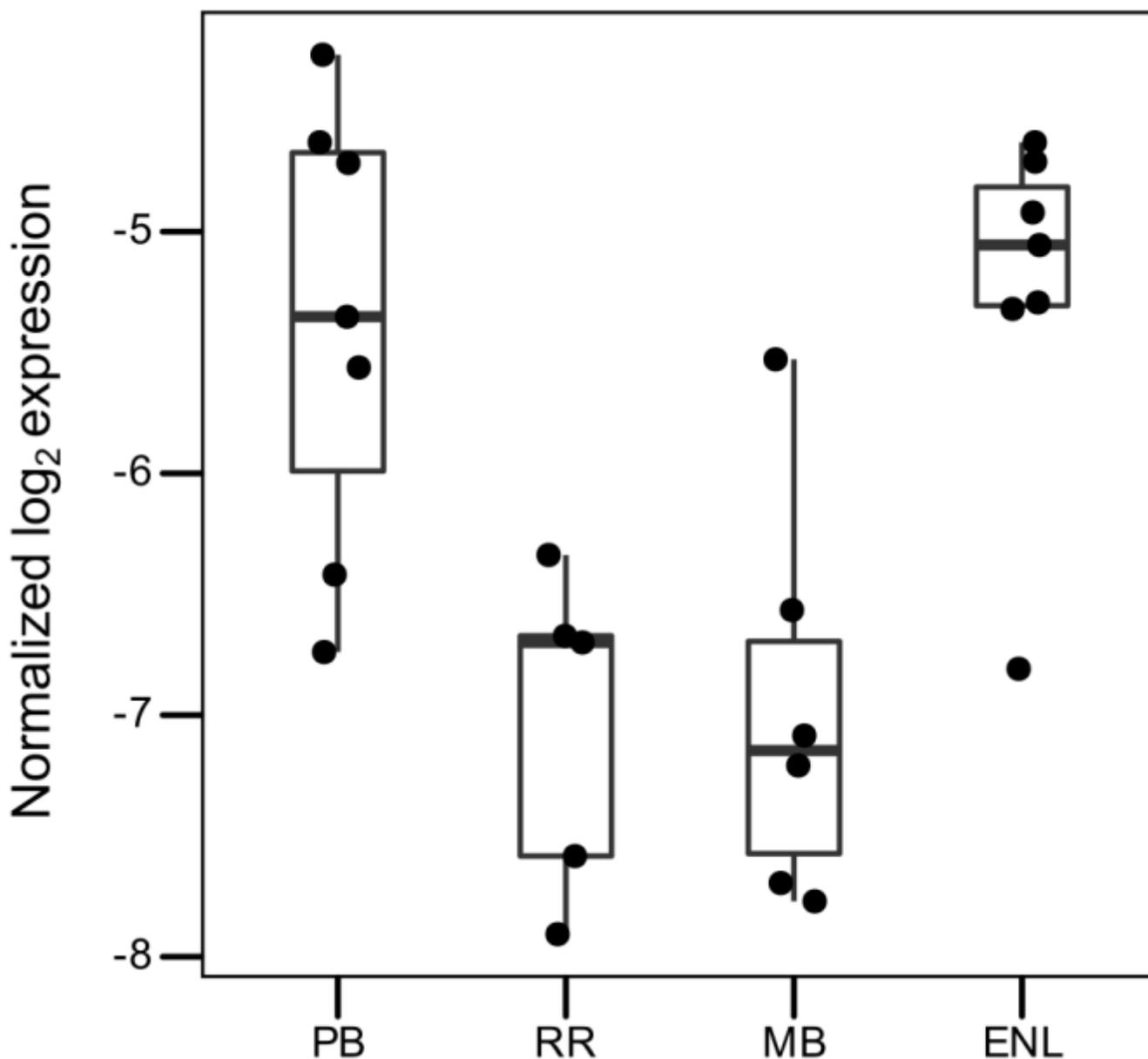


Figure 1

Normalized log2 expression of miR-125a-3p in leprosy patients. The logFC = log fold change, corresponds to a direct measure of how many times a gene is more or less expressed between groups. Data were analyzed using the Welch t test, through the R program, and considered significant if $P < 0.05$.

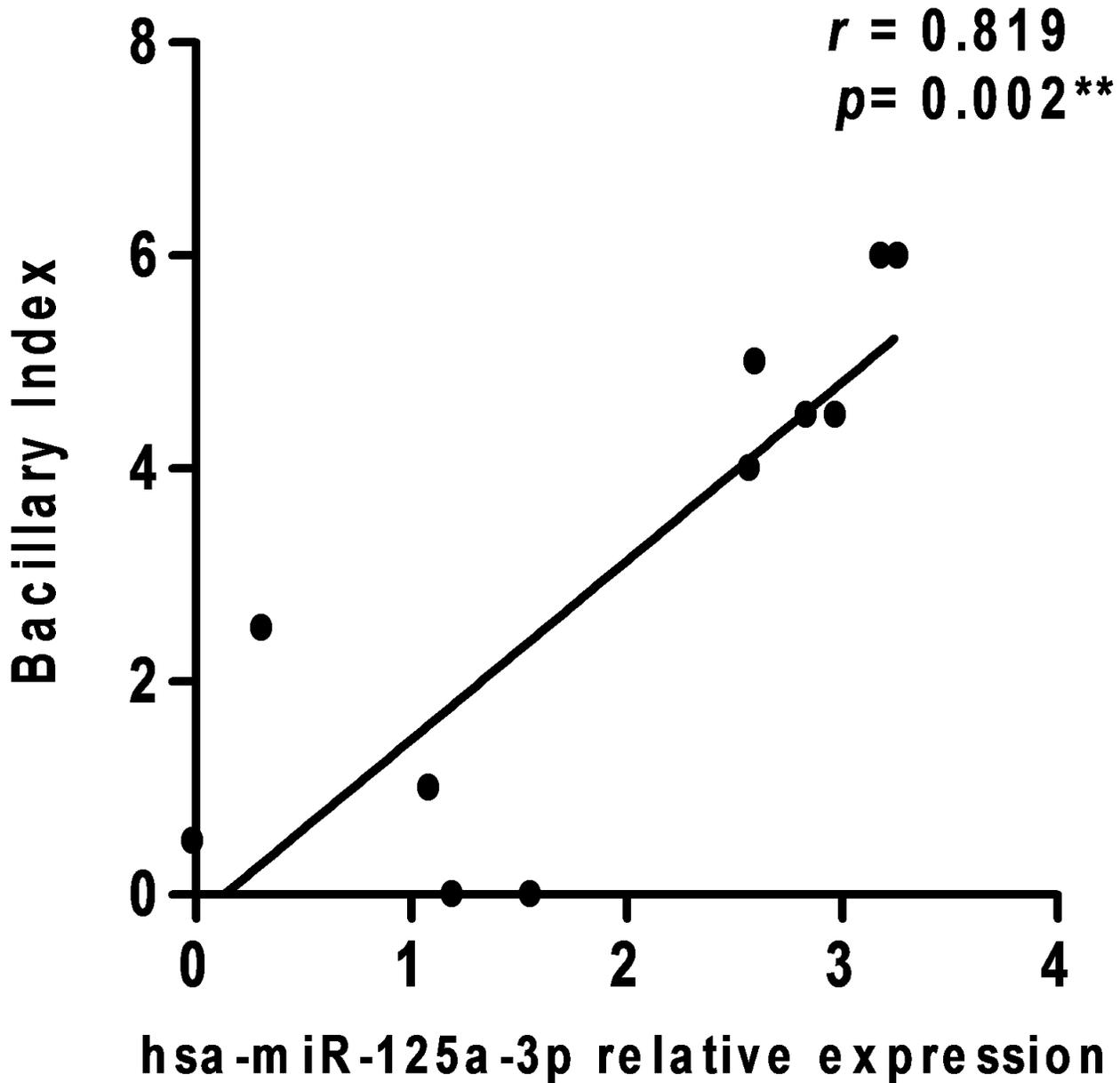


Figure 2

Positive correlation analysis between the bacillary index and the relative expression of miR-125a-3p in biopsy samples of reactional patients (RR + ENH). The data were analyzed by the Spearman Correlation test, using the GraphPad Prism software, where $r \geq 0.7$ was considered. Values of $p < 0.05$ was considered statistically significant.