Parasites & Vectors

LETTER TO THE EDITOR

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Microarray analysis of circular RNAs in HCT-8 cells infected with *Cryptosporidium parvum*

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Abstract

We read with great interest the article by Yin et al. (Parasit Vectors 14:238, 2021). The authors found that *Cryptosporidium* infection induced significantly aberrant expression of circular RNA profiles in HCT-8 cells, a finding which has far-reaching implications. However, due to the high number of false positives caused by multiple comparisons, statistical methods for microarray analysis should be carefully selected. Accurate analysis results will provide a convincing basis for subsequent experiments. In addition, we recommend several more appropriate methods in this article.

Keywords: *Cryptosporidium parvum*, Statistics, Bioinformatics, Microarray

To the Editor,

With the development of high-throughput microarray and RNA sequencing technology, an increasing number of genes have been identified to be associated with parasitology [1, 2]. We read with great interest the article by Yin et al. [3]. These authors, using microarray, found that *Cryptosporidium* infection induced significantly aberrant expression of circular RNA (circRNA) profiles in HCT-8 cells. Their findings provide a fundamental basis to develop effective strategies against *cryptosporidiosis* and, consequently, have far-reaching implications.

In our opinion, it is necessary to further clarify the data analysis strategy of this study. Based on the authors' description, they appear to use unadjusted *p*-values and fold change of expression values to define significantly differentially expressed circRNAs. However, due to the high number of false positives caused by multiple comparisons, statistical methods for microarray analysis should be carefully selected. Accurate analysis results will provide a convincing basis for subsequent experiments.

We suggest that the authors can adjust the *p*-values by the Benjamini–Hochberg correction to solve the problem of multiple comparisons, such as in the study of Atoyebi et al. [1]. Another option, as reported in a previous study, is linear modeling with empirical Bayes moderation, which provided good control of the false discovery rate as well as reasonable sensitivity when defining differentially expressed non-coding RNAs [4]. Therefore, we would like to suggest using Limma (linear models for microarray analysis) [5], which is an R/Bioconductor software package that uses linear models to analyze microarray and high-throughput PCR data [6]. Based on the output, the choice of the appropriate expression fold changes and false discovery rate < 0.05 as the cutoff is a conservative method to analyze changes in gene expression. A number of parasitological studies have used this statistical method and obtained satisfactory results [2]. We believe that this statistical method can provide a good technical support for parasitological studies.

We welcome further explanation of the data analysis strategy by the authors, which will make the results of this study more rigorous.

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Abbreviations

CircRNAs: Circular RNAs; Limma: Linear models for microarray analysis.

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YW and HZ conceived and prepared the first draft of the manuscript. LY and YZ critically reviewed the draft. All authors read and approved the final manuscript.

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References

1. Atoyebi SM, Tchigossou GM, Akoton R, Riveron JM, Irving H, Weedall G, et al. Investigating the molecular basis of multiple insecticide resistance in a major malaria vector *Anopheles funestus* (sensu stricto) from Akaka-Remo, Ogun State, Nigeria. Parasit Vectors. 2020;13:423.
2. Kim JW, Yi J, Park J, Jeong JH, Kim J, Won J, et al. Transcriptomic profiling of three-dimensional cholangiocyte spheroids long term exposed to repetitive *Clonorchis sinensis* excretory-secretory products. Parasit Vectors. 2021;14:213.
3. Yin YL, Liu TL, Yao Q, Wang YX, Wu XM, Wang XT, et al. Circular RNA ciRS-7 affects the propagation of *Cryptosporidium parvum* in HCT-8 cells by sponging miR-1270 to activate the NF-κB signaling pathway. Parasit Vectors. 2021;14:238.
4. Assefa AT, De Paepe K, Everaert C, Mestdagh P, Thas O, Vandesompele J. Differential gene expression analysis tools exhibit substandard performance for long non-coding RNA-sequencing data. Genome Biol. 2018;19:96.
5. Law CW, Alhamdoosh M, Su S, Dong X, Tian L, Smyth GK, et al. RNA-seq analysis is easy as 1-2-3 with limma, Glimma and edgeR. F1000Res. 2016;5:1408.
6. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 2015;43: e47.

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