Letter to Editor

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Commentary: Cross-talks between microbiota and microRNAs

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In recent years, the widespread physiological influence of microRNAs (miRNAs) has garnered immense interest given their demonstrated role in development, disease and regulation of gene expression. Investigation into the miRNA expression in the gut is a relatively new field, however a number of studies have demonstrated their involvement in intestinal architecture and function[1], cellular differentiation[2] and mucosal immunity[3]. Given their strength in numbers (~100 trillion) and well-established influence on host physiology - particularly by influencing intestinal epithelial cell gene expression - the potential for the gut microbiota to influence miRNA expression in the gut is intuitive. Indeed, recent investigations into this concept have suggested a role for the gut microbiota in eliciting miRNA expression changes throughout the gut [4-8]. A recent paper published in the mBio journal outlined the findings of a study into the miRNA expression response following oral *Listeria monocytogenes* infection between germ-free and conventional mice[9], and deduced that there was differential expression likely mediated through the gut microbiota. Although the study provides a worthy contribution to the nascent field of microbiota mediated miRNA expression in the gut, certain aspects of the study and the representation of the results warrants comment.

In the presented study, an initial signature of the most highly expressed miRNAs in the murine ileum was determined in uninfected conventional and germ-free mice, and germfree and conventional mice infected with *L. monocytogenes* following 24 hours and 72 hours of infection. These miRNA profiles were determined using Illumina sequencing and from this, a signature of the 10 most highly expressed miRNAs was derived. Whilst the abundance of some of the least expressed miRNAs varied slightly between the six conditions, the authors suggested that regardless of infection, these miRNAs formed a signature of the most highly expressed miRNAs in the murine ileum, which were corroborated by their previous detection in the murine gastrointestinal tract, with the exception of miR-378. A minor point that should be noted is that the expression of miR-378 has been previously detected in the murine gastrointestinal tract ([1]McKenna) - albeit in the jejunum and through different sequencing technologies – and therefore its expression in the murine gut is not a novel finding of this study. Of more importance, however, are discrepancies between the sequencing data used to initially detect this signature, and RT-qPCR data, which was used to validate their expression in replicates of the six conditions. For example, the fold change in expression of miR-148a compared with uninfected conventional mice is decreased by around 2-fold at 24 hours post infection, however was undetected initially by sequencing. A similar discrepancy exists between the expression of mir-200b, which is reduced by almost 2-fold in conventional mice 72 hours post infection, however again is undetected by sequencing. Furthermore, the expression of miR-143 detected RT-qPCR is more than 2-fold decreased in conventional mice at 72 hours post infection, yet remains essentially unchanged in the sequencing data. Lastly, the expression of miR-194 is more than 2-fold decreased in conventional and germ-free mice at 72 hours post infection, yet remains unchanged in the sequencing data. Whilst cross-platform differences in detection exist and may become more evident when looking at particularly low abundance microRNA transcripts, discrepancies of 2-fold are large enough to leave the true value of expression in question. This is a point of discrepancy that should have been discussed by the authors.

Similarly, there are several discrepancies between mRNA microarray and qPCR expression data. Initially, a microarray discovery approach was used to assess transcriptome wide differential expression of the protein-coding genes under the six conditions. A subset of 16

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differentially expressed genes were derived and validated in replicate using qPCR. A number of the genes whose expression levels were validated by qPCR are contrary to the initial fold change differences observed across the six conditions as per the microarray data. Of these genes, there are certain conditions in which Ly6a, Nlr5, Parp9, Nt5e, Cyp2c55, Cubn, Lct, Aft3 and RasGEF1b are either significantly differentially expressed as detected by microarray, but not with qPCR, or were not detected to be significantly differentially expressed as per the microarray data but reach statistical significance when detected by qPCR. For example, the expression of Nlr5 as detected by microarray in conventional mice following 72 hours of infection is not significantly altered, however, there is a 5-fold increase in its expression as detected by qPCR. Similarly, whilst the expression of Aft3 in conventional mice following 24 and 72 hours of infection is decreased just over two-fold compared with the uninfected mice, there are no significant differences in its expression as detected by qPCR. Furthermore, there are a number of discrepancies between the levels of fold change differences in expression of certain genes between the two sets of data. For example, whilst the expression of Fut2 is 5-fold increased in germ-free mice following 72 hours of infection, this difference is almost 100-fold increased as detected by qPCR. Again, whilst cross-platform differences in gene expression detection are to be expected, there are some large discrepancies between the microarray and qPCR data that have not been discussed by the authors and may be important in appreciating the true influence of the gut microbiota on the expression of these genes.

Following from this, the representation of the miRNA and mRNA expression RT-qPCR data in this way is not ideal and potentially obscures the biological relevance of the expression changes. Whilst fold change in expression between the uninfected and infected conditions gives information on the relative differences, there is no information given as to the magnitude of target transcript and therefore potentially leaves the biological relevance of these differences in question. As discussed by Hardikar and colleagues[10], a more determinative way to represent RT-qPCR data is to display data as fold over detectable of the raw CT values, allowing evaluation of the transcript abundance. Furthermore, representation of the data in this way would enable direct comparison with the miRNA sequencing data, as in both cases the relative abundance of transcripts could have been appreciated.

Lastly, the extent to which the gut microbiota mediate the expression of certain miRNAs and protein-coding genes is potentially obscured by the design of the study, given that differences were assessed between germ-free and conventional mice. There are several inherent and environmental differences - such as the histology, anatomy and physiology of the gut and other body systems, nutritional requirements and housing etc. - that differ between germ-free and conventional mice, that may influence the response to *L. monocytogenes* infection beyond the gut microbiota alone. These factors have not been controlled for in this study. An alternative method of investigation that may dispel doubt as to any confounding variables would be to investigate the miRNA/mRNA response following *L. monocytogenes infection in* germ-free mice, and in germ-free mice colonized with the bacteria from pathogen free conventional mice. This would remove the bias in differences between germ-free and conventional mice and allow an assessment of the isolated effect of the gut microbiota on miRNA/mRNA expression following infection. As such, although the data suggest gut microbiota mediate the expression of a number of miRNAs/mRNAs, it cannot be concluded that the gut microbial presence alone is the sole reason for this.

In summary, whilst the work of Archambaud and colleagues suggests an involvement of the gut microbiota in mediating the expression of certain miRNAs and protein-coding genes in response to *Listeria monocytogenes* infection, there are certain aspects of the study and presentation of results that potentially limit better appreciation of these findings. Specifically, there are a number of discrepancies in the miRNA and mRNA expression data as detected by sequencing and microarray respectively and with the validating RT-qPCR data. Given that a number of these discrepancies are large, their impact on the overall findings of the study should have been discussed. Furthermore, variables between conventional and germ-free mice could have been controlled for using an alternative experimental approach to determine the isolated effect of the gut microbiota. Nonetheless, the authors provide the first assessment of the role of the intestinal microbiota in the regulation of miRNA and protein-coding gene expression in the host following *Listeria monocytogenes* infection. Given the results of this study suggest a mediating role for the gut microbiota in eliciting differential miRNA and mRNA expression, this study adds to the mounting evidence that the gut microbiota hold an influential role in gut miRNA expression.

Conflict of interest statement: Authors state no conflict of interest

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