(Form 1)

Kyoto University	Doctor of Philosophy in Life Sciences	Name	Lin, Hsien-Hen
Thesis Title	Investigation of Innate Immune Responses in *Eptesicus* Bat Cells via Comprehensive Analysis		

(Thesis Summary)

Bats, the order *Chiroptera*, are known to coexist with many viruses without obvious symptoms and are thought to serve as reservoir hosts for various zoonotic pathogens. It has been hypothesized, therefore, that bats have a unique immune system against viral and other pathogen infections and may be able to easily establish a coexistence with many viruses. However, analysis of the immune system of bats is limited and much remains to be clarified. This study comprehensively analyzed gene expression profiles of innate immune responses using cell lines derived from the genus *Eptesicus*.

In this study, two types of *Eptesicus* bat kidney-derived cell lines (Efk3B and EnK cells) were treated with poly(I:C) and mRNA-seq was performed to analyze the gene expression profiles involved in the innate immune response in detail. The results showed that the gene expression profiles of the two cell lines after poly(I:C) treatment were similar, but there were some gene groups that were differentially expressed in each cell line. In addition, the genes upregulated in response to poly(I:C) induction were analyzed in comparison with those in a human-derived cell line, HeLa cells. The results showed that the upregulated genes of *Eptesicus* bat cells were distinct from those of human cells in response to induction and also that the upregulation was particularly concentrated in the interferon-gamma pathway in bat cells. In addition, the expression of selected interferon-stimulated genes, including MX1 and IFIT1, in Efk3B and EnK responded more rapidly to universal type I IFN treatment than to poly(I:C). Furthermore, the basal expression levels of several immune-inducible genes, such as IFIT2 and IFIT3, which are hub genes of the interferon pathways, were found to be high even in the normal state without induction, indicating that bat cells may be in a steady-state of immune activation. These results indicate that the innate immune response in *Eptesicus* bat cells is different from that in human cells.

Moreover, in this paper, the expression profiles of transposable elements (TEs) and microRNAs (miRNAs) were analyzed in both *Eptesicus* bat cell lines after poly(I:C) treatment. As a result, TE elements and miRNAs whose expression has not been previously found to vary in mammalian cells were identified, suggesting that the innate immune response of *Eptesicus* bat cells is affected by different pathways through a yet unknown mechanism.

This is the first study to reveal the gene expression profiles, including mRNAs, TEs, and miRNAs, of the innate immune responses in *Eptesicus* bat cells. The data provide basic and innovative insights into bat innate immunity as well as represent a valuable resource for future research into bat immune systems and the biology of *Eptesicus* bats.

(Form 2)

(Thesis Evaluation Summary)

Bats have been reported to be the host of various zoonotic viruses, including coronaviruses, Ebola virus, rabies virus, and Nipah virus. Therefore, bats are thought to have a unique immune response system against viruses, but the details have not been clearly understood yet.

In this study, the candidate used two types of line cells derived from *Eptesicus* bats (Efk3B and EnK cells) to comprehensively analyze the gene expression profile involved in the innate immune response. There are few studies similar to the present work, and this study is significant in the understanding of the immune system of bats.

Using *Eptesicus* bats cell lines treated with poly (I:C) or type I IFNs, the candidate comprehensively analyzed gene expression profiles by mRNA-seq and small RNA-seq analyses. The results showed that while the two bat cell lines showed similar gene expression profiles, they also showed genes with specific expression patterns in each cell type, indicating that closely related species have different gene expression profiles. Furthermore, the genes up-regulated by poly(I:C) were found to be different between bat cells and human-derived cells, demonstrating the specificity of the innate immune response in *Eptesicus* bat cells. The candidate also identified various expression altered genes, which had not been shown in previous studies, and successfully discussed the significance of the expression of such genes. Furthermore, this study found that the expression of certain IFN-responsive genes is high in bat cells even in the absence of poly (I:C) induction, indicating that bat cells may be in an immunologically active state even in the steady state. This finding is extremely important in considering the coexistence of bats and viruses.

In addition, the candidate has analyzed the alterations of the expression of transposable elements (TEs) and microRNAs (miRNAs) in bat cells and showed unique expression profiles of TEs and unknow miRNAs, which have not been demonstrated in previous studies, demonstrating a new perspective on the innate immune response of bat cells.

This is the first report of a comprehensive analysis of the innate immune response in the *Eptesicus* bats. The results of this study will provide valuable insights not only into the immune system of bats, but also into the biology of *Eptesicus* bats.

This thesis substantiates the candidate's extensive and wide knowledge of life sciences, demonstrates expert research capability in the fields of bioinformatics and immunology, and presents new discoveries and concepts that contribute to the profound understanding and further development of the candidate's research field. Moreover, the thesis is written logically and coherently, which satisfies the degree requirement that the thesis shall serve as a valuable document for future reference. On February 2nd, 2022, the PhD thesis oral examination was held. Pursuant to this oral examination, the thesis examination committee hereby concludes that the candidate has passed all of the requirements for the degree of Doctor of Philosophy in Life Sciences.

The thesis, thesis summary, and thesis evaluation summary will be published through the Kyoto University Research Information Repository. If the thesis cannot be published on the website immediately after the degree is awarded, due to patent application, journal publication constraints, or other reasons, please indicate the earliest date that the thesis can be published. (Please note, however, based on Article 8 of the Degree Regulations, that the thesis must be published within three months of the date that the degree is awarded.)

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