Cell Biol Toxicol
https://doi.org/10.1007/s10565-022-09771-9

SHORT COMMUNICATION

Comparative analysis of single cell lung atlas of bat, cat, tiger, and pangolin

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Received: 21 January 2022 / Accepted: 26 August 2022

Abstract Horseshoe bats (*Rhinolophus sinicus*) might help maintain coronaviruses severely affecting human health, such as severe acute respiratory syndrome coronavirus (SARS-CoV). Bats may be more tolerant of viral infection than other mammals due to their unique immune system, but the exact mechanism remains to be fully explored. During the coronavirus disease 2019 (COVID-19) pandemic, multiple animal species were diseased by coronavirus infection, especially in the respiratory system. Herein, a comparative analysis with single nucleus transcriptomic data of the lungs across four species, including horseshoe bat, cat, tiger, and pangolin, were conducted. The distribution of entry factors for twenty-eight respiratory viruses was characterized for the four species. Our findings might increase

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Highlights
- A comparative analysis using single nucleus transcriptomic data of the lungs across four species (horseshoe bat, cat, tiger, and pangolin) was conducted.
- The distribution of entry factors for twenty-eight respiratory viruses was characterized for the four species.
- Comparison on the immune-related transcripts might increase our understanding of the immune background of horseshoe bats.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10565-022-09771-9.

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Published online: 28 September 2022

our understanding of the immune background of horseshoe bats.

Keywords Single cell · Bat · Cross species · Comparative analysis

Bats are important reservoir hosts for a myriad of viruses. Novel bat coronaviruses were identified via meta-transcriptomic investigation of hundreds of bat samples (Zhou et al. 2021). The immune response induced by virus infection was shown to differ between human and bat cells (Glennon et al. 2015; Wynne et al. 2014) and that bats may have their unique transcripts that are not present in other mammals (Papenfuss et al. 2012). Bats were found to have limited interferon activation due to mutation in the STING protein (Xie et al. 2018) and have contracted type I IFNα locus but constitutive IFNα expression without viral stimulation (Zhou et al. 2016). The unique immune response pathways and antiviral gene expression profile of bats may promote their tolerance to viral infections (Irving et al. 2021). Although the direct progenitor of SARS-CoV-2 remains unknown, its closest relative (RaTG13) has been detected in a horseshoe bat (*Rhinolophus sinicus*), indicating horseshoe bats as its potential reservoir hosts. Moreover, horseshoe bats were also found to harbor other groups of coronaviruses including the SARS-CoV (Ge et al. 2013; Hu et al. 2017; Li et al. 2005), indicating their critical role in the maintenance of human sensitive coronaviruses. Since the outbreak of COVID-19, multiple animal species have been infected and diseased by coronavirus, including pangolins, cats, tigers, etc. (Lam et al. 2020; Liu et al. 2019; McAloose et al. 2020; Newman et al. 2020; Zhang et al. 2020). Herein, we constructed the single-nucleus atlas of bat lung tissues and conducted a comparative study to elucidate the lung immune landscape of bat, cat, tiger, and pangolin (Chen et al. 2022; Chen et al. 2021), which might help reveal the molecular basis for their differential immune behaviors upon infections by coronaviruses.

Due to species-specific immune response upon viral infection, clinical symptoms in the lower respiratory differ among species. While bats, cats, tigers, and pangolins were all permissive to coronavirus infection, details of their biological background remain unknown. Herein, we collected lung tissues from two individuals of bats to generate single-nucleus libraries of lung cells, resulting in a total of 11, 838 pulmonary cells passing quality control (Fig. 1a, b, Fig. S1a, b, Table S1). Nine major cell types were identified in the lung atlas of bats, which included alveolar type 1 cells (AT1), alveolar type 2 cells (AT2), ciliated cells, secretory cells, endothelial cells, fibroblasts, T cells, B cells, and macrophages, each demonstrating the specific expression of canonical cell type markers (Fig. 1c, Table S2).

Receptor binding is critical for viral entry into cells and that the distribution of receptors reveals the susceptibility of cells to viral infection, which may further stimulate the local immune response. Here, we determined the expression patterns of 29 genes encoding entry factors of respiratory viruses in the lung cells of the four species. *Itgb5* (a receptor of adenoviruses) and *Anpep* (a receptor of human coronavirus 229E) were highly enriched in bat AT1 and AT2, respectively. Another adenovirus receptor, *Cd86*, was enriched in the macrophages of bat, pangolin, and tiger. The rhinovirus receptor, *Cdhr3*, was enriched in the ciliated cells of bat, cat, and tiger. Adeno-associated virus receptor *Rpsa* was significantly expressed in tiger lung cells. Marginal expressions of *ACE2* were observed in bat ciliated cells. However, the entry factor for SARS-CoV-2, *Scarb1*, displayed high expression in bat endothelial cells and macrophages. Another two SARS-CoV-2 entry factors, *Nrp1* and *Axl*, also showed significant cell type and species specificity. *Nrp1* was largely enriched in AT1/AT2 of tigers and AT2 of bats, whereas *Axl* was highly expressed in fibroblasts and macrophages of bat lung and fibroblasts of pangolin lung (Fig. 1d). Cytokine storm, due to excessive and uncontrolled release of pro-inflammatory cytokines, is one of the main culprits contributing to severe lung pathogenesis caused by various virus infections (Tisoncik et al. 2012). As a natural reservoir for zoonotic viruses, bats display no significant symptoms after virus infection due to its unique immunity (Banerjee et al. 2020). Here, we compared the expression profiles of a variety of cytokines among distinct pulmonary cell types of bat, cat, pangolin, and tiger (Figure S2). *Lif* plays important roles in several inflammatory disorders (Gadient and Patterson 1999) and was lowly expressed in bat. *Osmr* was abundantly expressed on mice pulmonary endothelial and fibroblast cells. We observed enrichment of *Osmr* in these two cell types of cat and tiger,

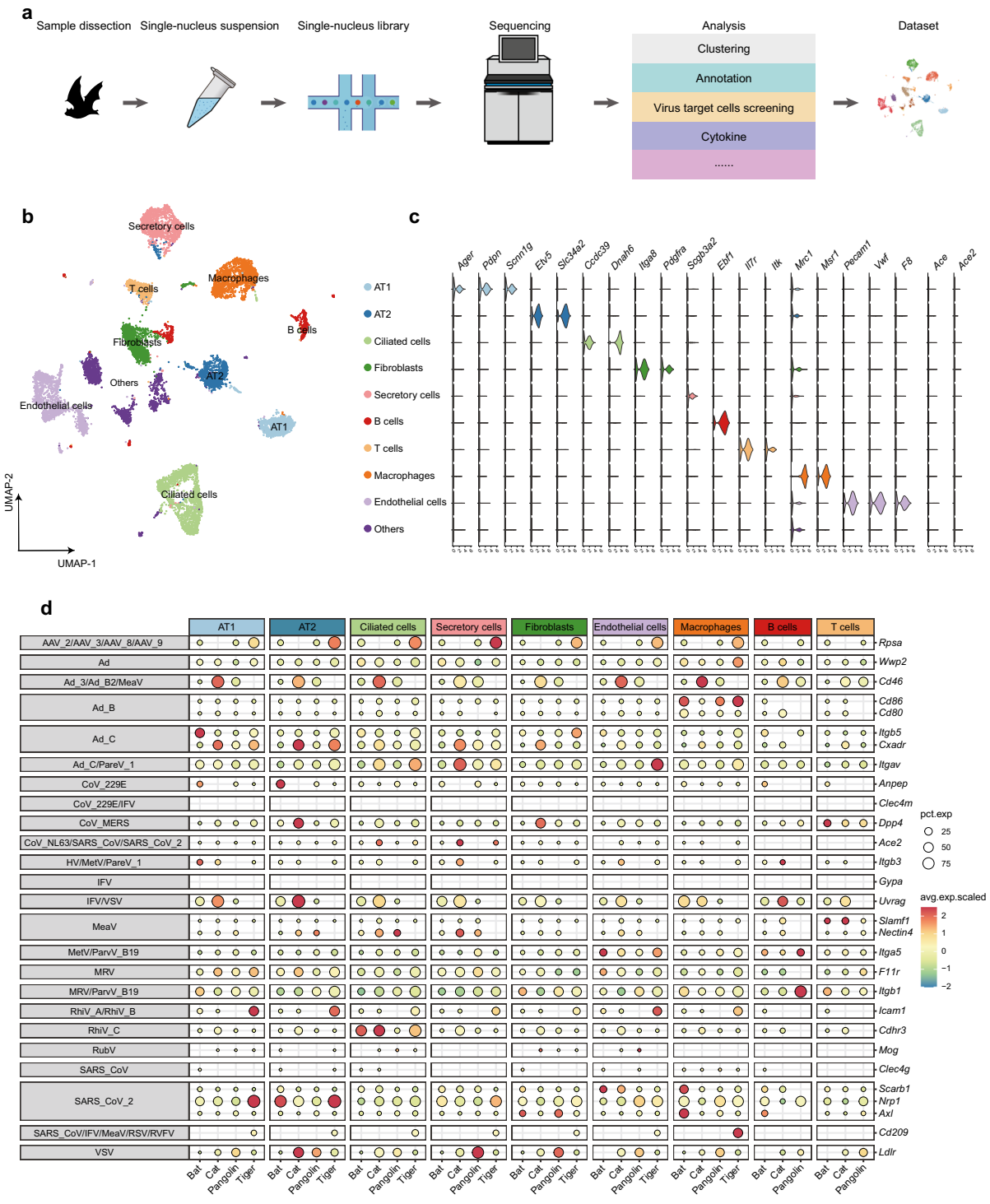

Fig. 1 Comparative single nucleus lung atlas of bat, cat, tiger, and pangolin. **a** Illustration of the overall project design. **b** Uniform Manifold Approximation and Projection (UMAP) plot of bat lung single cell atlas. **c** Violin plot showing the expression patterns of canonical cell type markers. **d** Expression proportion and scaled expression value of virus receptors in distinct cell types of bat, cat, tiger, and pangolin

and wide expressions in all the nine pulmonary cell types of pangolin.

In this study, we have generated the single nucleus transcriptomes for the lungs of horseshoe bats. Our transcriptome data revealed their cellular heterogeneity and thus laid the foundation for in-depth comparative study regarding the cellular and immune biology upon virus infections. Due to experimental limitations, we have only characterized the cytokine expressions of the lung cells of bat, cat, tiger, and pangolins, which may help unravel the baseline expression of immune factors. To fully understand immune responses stimulated by specific viral infections in bats, transcriptome data from appropriately controlled infection experiments are desired to better illustrate the differential pulmonary immune responses between these species. Moreover, because bats, tigers, and pangolins were feral species, the animals sampled may not be strictly healthy as the pathogen-free laboratory animals. It should also be noted that we only characterized gene expression at transcriptome level, losing information such as post-transcriptional regulation and protein modification. Considering that transcript level might not be positively correlated with protein level in some occasions, the conclusion drawn in this work needs to be confirmed by experiments in further studies.

Author contribution All authors contributed to the study conception and design. Dongsheng Chen, Jian Sun, Huan Liu, and Xiran Wang conceived and designed the project. Xiran Wang was responsible for sample collection and dissection. Chengcheng Sun and Wendi Wu participated in single-nucleus library construction and sequencing. Xiran Wang, Peiwen Ding, Daxi Wang, Jiacheng Zhu, Meiling Li, Xin Jin, and Wensheng Zhang coordinated data analysis. Xiran Wang and Peiwen Ding contributed to single-cell clustering and cell annotation. Xiran Wang, Peiwen Ding, Chengcheng Sun, Rong Xiang, Yanan Wei, Xiangning Ding, and Lihua Luo participated in data interpretation, visualization, and manuscript writing. All authors read and approved the final manuscript.

Funding This work was supported by the Guangdong Major Project of Basic and Applied Basic Research (Grant 2020B0301030007), the Local Innovative and Research Teams Project of Guangdong Pearl River Talents Program (Grant 2019BT02N054), the Program for Changjiang Scholars and Innovative Research Team in University of Ministry of Education of China (Grant No. IRT_17R39), the Innovation Team Project of Guangdong University (Grant No. 2019KCXTD001).

Data availability Raw transcriptome sequencing data sets were deposited at the CNSA (CNP0002166).

Code availability Scripts used in this study are available at https://github.com/retroplay/Bat-Lung-scRNA-seq.

Declarations

Ethics approval Sample collection and research were performed with the approval of Institutional Review Board on Ethics Committee of BGI (approval letter reference number BGI-NO. 20140-T1).

Consent to participate Not applicable.

Consent for publication All authors have given consent to publication of the manuscript.

Conflict of interest The authors declare no competing interests.

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