EAACI Research Fellowship – Final Report

Name: Kai Sen Tan

Project title: Abnormal energy profile of inflamed nasal epithelium affecting ciliary assembly and function as mechanism of secondary ciliary dyskinesia

Type of Fellowship: Medium Term Research Fellowship

Duration: 1st September 2019 – 28th February 2020 (6 months)

Location: Group of Prof. Kian Fan Chung, National Heart and Lung Institute, Imperial College London, London, UK

What questions were addressed and why?

The airway epithelium and its surrounding structural cells is the primary site of pathology for chronic airway inflammatory diseases. The concerted effort of the airway cells ensured the proper functioning of the airway physiological functions, as well as preventing damage leading to any impairment[1]. However, in many chronic inflammatory airway diseases elicited by either allergic or infective (viral or bacterial) processes, loss of function or dysfunction in airway physiology leads to the pathology and unresolved inflammation in the airway leading to chronic inflammatory symptoms[2-4]. While proper ciliary ultrastructure and/or function is indispensable to maintain the homeostasis of the airway, this has been often overlooked as an important target for intervention in the treatment of chronic airway inflammatory diseases. We previously showed that ciliary ultrastructural defects are associated with chronic airway inflammatory diseases[5,6]. As Such, functional restoration of defective respiratory cilia represents a novel approach which could act synergistically with other anti-inflammatory approaches[7,8].

What was the nature of the research?

To address the questions pertaining to ciliary impairment and their association to chronic airway inflammatory diseases, identification of the potential mechanisms underlying defective cilia (including indirect causes such as airway microenvironment changes, altered homeostasis and metabolism and differentiation changes) if required. Such work will lead to the development of novel and effective approaches for reversing and controlling the persistence and evolution of inflammatory responses in chronic airway diseases. Hence, -omics based research was used to identify potential mechanisms, and laboratory research was carried out to verify the mechanism involved based on the following **hypothesis**: Inflammation in the airways with chronic inflammatory diseases alters airway metabolism and energy dynamics in the epithelium and surrounding structural cells that may contribute to ciliary changes and chronic inflammatory pathologies.

Research course adapted from the original plan

While the hypothesis remained the same, changes have been adopted for the research plan due to the duration of the fellowship and the decision to focus the resources on cilia associated changes. To accomplish this, a list of motile cilia related genes was collated from previous studies and Gene ontology database to identify the main ciliary dysfunction and associated impairments in chronic airway inflammatory diseases. The list was analyzed in U-BIOPRED dataset to establish ciliary dysfunction in asthmatic airways. The functional impairment of motile cilia can then be applied to functional and studies to identify the mechanisms of ciliary dysfunction in chronically inflamed airway. In addition, due to resource constrains on the cell types banked from volunteers, airway smooth muscle cells (ASMC) were used as a proxy for the experiment on airway cell energy state to establish the seahorse protocol for future experiments on the airway epithelium. With these changes, the specific aims were adapted to the following:

Specific aim 1: To identify motile cilia and associated dysfunctions and their prevalence in asthmatic phenotypes under inflammatory conditions.

Specific aim 2: To compare energy state and mitochondrial functions of airway smooth muscle cells between healthy and asthmatic airways as a means of establishing seahorse assay protocol for airway epithelium comparison.

Research performed pertaining to specific aims:

Establishment of genes involve in the formation and function of motile cilia

As there are currently not many studies focusing on motile cilia and their role in chronic airway inflammatory diseases, we first establish a list of genes involved in the formation and function of motile

cilia, including ciliogenesis, ciliary assembly and cilia motility. By identifying ciliary genes from experimental evidences and online databases, we collated a gene list of 269 genes that were involved in different aspects of motile cilia formation and functions.

Motile cilia gene expression impairment in U-BIOPRED asthmatic cohorts
Upon establishing the ciliary gene sets, gene set variation analysis (GSVA) was performed on with the motile cilia gene sets, its sub-annotations and associated functions like ATP production and respiration on U-BIOPRED datasets of asthma patients. By comparing the enrichment levels of the motile cilia and associated gene sets in nasal brushings and sputum cells of severe and mild asthmatics to healthy controls, we identified and listed potential ciliary and associated functions linked to asthma severity and pathogenesis. The functions most impacted include ciliogenesis, ciliary assembly and functions. In additoon, we also further look at the ATP production in these datasets as ciliary assembly and functions are ATP intensive functions. There is a significant degree of overlap between ATP synthesis and oxidative phosphorylation suggesting that the impairment of the ATP production gene expression may arise from the mitochondria, in which oxidative phosphorylation to generate ATP occurs.

ATP synthesis and production in airway smooth muscle cells from donor sources of different asthmatic severity
In order to further ascertain that ATP production may be altered in asthmatic airway cells, we subjected ASMC from severe asthma, mild and moderate asthma, and healthy control donors to seahorse assay to assess the ATP production and ATP-linked respiration. This was done using Seahorse XF ATP-Rate assay and Mito Stress assay kits in the Seahorse analyzer to assess oxygen consumption rate (OCR) for the calculation of ATP production rate, basal respiration and ATP-linked respiration. ASMCs from different donor source were cultured and analyzed for their ATP production. The comparison showed that the rate of ATP production from both glycolysis and mitochondrial pathway, as well as ATP-linked respiration may be affected in an asthmatic airway as shown in ASMC. This warrants further increase in experimental numbers as well as testing in other cells to assess if the trend and difference persist across different cell types in the airways. Additionally, we also further found that induction of the ASMCs into inflammatory (TNFα) and proliferative (TGFβ with serum) states further altered the ATP production rate and ATP-linked respiration.

How will the findings impact future research?
In this 6-month research fellowship, we investigated the role of motile cilia and the mechanisms of its impairment in chronic airway inflammatory disorder based on understanding of our previous work. We studied the motile ciliary gene expression in U-BIOPRED cohorts to identify potential genes from the nasal brushing and sputum on motile cilia that may function as biomarkers for asthmatic airways, as well as to find out potential mechanisms to focus on for studying of motile cilia impairment. We identified that the sputum cells contained source of mis-expressed motile cilia genes and that the main impairment in motile cilia is due to aberrant expression of ciliogenesis, ciliary assembly and function genes. In addition, we also assess the energy state of airway cells using ASMCs through Seahorse assay and found reduced ATP production rate that may indirectly contribute ATP poor environments in the airway but would require further studies on epithelial cells to confirm such findings. Therefore, the future work for the continuation of this study is to investigate the energy state of basal cells from the nasal epithelium, as well as the fully differentiated nasal epithelial cells using Seahorse assay. The investigation of the submerged basal cell culture can be directly performed on the Seahorse analyzer and they can be compared between basal cells of different sources. Fully differentiated nasal epithelial cells in air-liquid interface (ALI) would require specialized well in Seahorse which require further optimization for that portion of the study.

Personal reflection on the time spent for the fellowship
The 6 months spent in Fan Chung's lab in NHLI, Imperial College London is a fruitful one. While there were needs to adapt the research protocol due to the changes in resources available, the situation is a good learning experience as it trains people involved to be able to best make use of available resources to achieve the best outcome in the study. Throughout the fellowship, I have learned new techniques like seahorse that is an important component for the motile cilia studies that I am pursuing. Additionally, learning GSVA has equipped me with an additional analysis tool for integrating transcriptomics and other large omics dataset to assist in my research studies. At he same time, the work performed can be followed up and may lead to publishing of 1 to 2 scientific article in the field. Finally, the people I met and collaborated in Imperial College London expanded my network and I look forward to building more collaborations in the future as I pursue an academic career. On the other hand, areas which I sought to

improve is to engage in more detailed planning in understanding the resources available to prevent time loss in adapting plans and experiments. Furthermore, I would also like to learn more on prioritizing the most important aspects of the learning experience when presented with a variety of learning opportunities. Being focused on the most prioritized portion of the opportunities would enable me to stay focus on my goals in my career and would help me in the long run as there will be times where I will have to choose and will be unable to handle everything presented. In conclusion, the fellowship has equipped me further with the tools I need to succeed in my pursuit of a scientific career.

Acknowledgement

I would like to thank the host of the fellowship, Professor Kian Fan Chung in Imperial College London for the opportunity to perform research in an overseas Institute. I would also like to express my gratitude to Dr Pankaj Bhavsar, who invited me to join his ongoing study for the wet lab portion of the fellowship in which he provided the resources and guidance for the Seahorse assays. In addition, I would also like to thank other PIs in the institute, Professor Ian Adcock and Professor Sebastian Johnston, who provided scientific advice and assistance during my stay there. I am also thankful to the research fellows and students which make my time in the lab full of learning opportunities and enjoyment, particularly Dr Sharon Mumby, Ms Julia Garcia and Dr Eva Delbrel. I would also like to give thanks to the other visiting academics Professor Song Woo Jung, Dr Serena Xu and Dr Sherry for the exciting pursuit of scientific discovery in the lab. I would also like to express thanks to the laboratory technicians and administrative staff Ms Lisa Ingram, Ms Carolyn Green and Ms Jacquie Ujetz who helped me settle into the lab. My gratitude also goes to Dr Nazanin Zounemat Kermani and Mr Badi Yusef from Data Science Institute in helping me with the omics analysis and in continually keeping in contact even beyond the fellowship. I would also like to thank each and every one that I met in the institute during my fellowship for making my stay as fruitful and comfortable as possible. Last but not least, my sincerest gratitude to my fiancée Rachel Lim who gave her most earnest support when I am overseas completing the fellowship.

List of publications during fellowship period related to the study

1. **Tan KS**, Lim RL, Liu J, Ong HH, Tan JY, Lim HF, Chung KF, Adcock IM, Chow VT and Wang DY. (2020) Respiratory viral infections in exacerbation of chronic airway inflammatory diseases: Novel mechanisms and insights from the upper airway epithelium. Front Cell Dev Biol. https://doi.org/10.3389/fcell.2020.00099. **First author** (IF: 5.206)
2. Zhou M, **Tan KS**, Guan WJ, Jiang LJ, Deng J, Gao WX, Lee YM, Xu ZF, Luo X, Liu C, Shi JB, Lai Y. (2020) Proteomics profiling of epithelium-derived exosomes from nasal polyps revealed signalling functions affecting cellular proliferation. Respir Med. 162: 105871. (IF 3.237)
3. Zi XX, Guan WJ, Peng Y, **Tan KS**, Liu J, Shi L, Wang DY. (2019). An integrated analysis of radial spoke head and outer dynein arm protein defects and ciliogenesis abnormality in nasal polyps. Front Genet. *10: 1083*. (IF 3.517)

References

1. Bustamante-Marin, X.M. & Ostrowski, L.E. Cilia and Mucociliary Clearance. *Cold Spring Harb Perspect Biol* **9**(2017).
2. Lai, Y., Chen, B., Shi, J., Palmer, J.N., Kennedy, D.W. & Cohen, N.A. Inflammation-mediated upregulation of centrosomal protein 110, a negative modulator of ciliogenesis, in patients with chronic rhinosinusitis. *J Allergy Clin Immunol* **128**, 1207-1215 e1201 (2011).
3. Li, Y.Y., Li, C.W., Chao, S.S., Yu, F.G., Yu, X.M., Liu, J.*, et al.* Impairment of cilia architecture and ciliogenesis in hyperplastic nasal epithelium from nasal polyps. *J Allergy Clin Immunol* **134**, 1282-1292 (2014).
4. Thomas, B., Rutman, A., Hirst, R.A., Haldar, P., Wardlaw, A.J., Bankart, J., Brightling, C.E. & O'Callaghan, C. Ciliary dysfunction and ultrastructural abnormalities are features of severe asthma. *J Allergy Clin Immunol* **126**, 722-729 e722 (2010).
5. Peng, Y., Guan, W.J., Tan, K.S., Zhu, Z., Chen, Z., Hong, H.*, et al.* Aberrant localization of FOXJ1 correlates with the disease severity and comorbidities in patients with nasal polyps. *Allergy Asthma Clin Immunol* **14**, 71 (2018).
6. Qiu, Q., Peng, Y., Zhu, Z., Chen, Z., Zhang, C., Ong, H.H.*, et al.* Absence or mislocalization of DNAH5 is a characteristic marker for motile ciliary abnormality in nasal polyps. *The Laryngoscope* **128**, E97-E104 (2018).
7. Li, Y.Y., Liu, J., Li, C.W., Subramaniam, S., Chao, S.S., Yu, F.G., Cohen, N.A., Li, S. & Wang, Y. Myrtol standardized affects mucociliary clearance. *International forum of allergy & rhinology* **7**, 304-311 (2017).
8. Butler, C.R., Hynds, R.E., Gowers, K.H., Lee Ddo, H., Brown, J.M., Crowley, C.*, et al.* Rapid Expansion of Human Epithelial Stem Cells Suitable for Airway Tissue Engineering. *Am J Respir Crit Care Med* **194**, 156-168 (2016).