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LETTER TO THE EDITOR

Distinct stem/progenitor cells proliferate to regenerate the trachea, intrapulmonary airways and alveoli in COVID-19 patients

Cell Research (2020) 30:705–707; https://doi.org/10.1038/s41422-020-0367-9

Dear Editor,

The global pandemic COVID-19 caused by SARS-CoV-2 virus has infected over 6.5 million individuals and claimed over 350,000 lives worldwide within six months. The respiratory epithelial cells covering the airways and alveoli are the major targets of the virus. Moreover, damage to the epithelium can be exacerbated by mechanical ventilation. It is expected that many of the infected individuals that survived the acute phase will develop pulmonary diseases (e.g. fibrosis) if the epithelium fails to regenerate properly.

Multiple stem/progenitor cells have been implicated in the regeneration of the respiratory epithelium. The human trachea and intrapulmonary airways are lined by three major cell types, basal, club and ciliated cells, and the alveolar epithelium includes type 1 (AT1) and type 2 (AT2) cells. The trachea basal cells have been shown to serve as progenitor cells to self-renew and differentiate into other cell types including club, ciliated cells and minor cell populations (e.g., tuft cells).[1,2] Club cells can also de-differentiate into basal cells to regenerate the tracheal epithelium in a mouse model where basal cells are ablated prior to injury.[3] In the intrapulmonary airways, basal cells have been postulated to serve as progenitor cells for epithelial regeneration in humans (reviewed by[4]). In mice, however, club cells are responsible for repopulating the intrapulmonary airway epithelium upon injury due to their lack of basal cells in the intrapulmonary airways.[5] The cell of origin for regenerating the alveolar epithelium remains controversial. Multiple cell types have been implicated in the regeneration of the alveolar epithelium depending on injury models. These cells include AT1 and AT2 cells, bronchial-alveolar ductal cells (BASCs), distal airway stem cells (DASCs), lineage negative epithelial precursor (LNEP) cells, bronchial epithelial stem cells (BESCs), and different AT2 subpopulation cells (reviewed by[6]). Although histology analysis has been performed, it remains unknown which stem/progenitor cell(s) proliferate in response to viral challenges in COVID-19 patients.

Cellular entry of SARS-CoV-2 depends on the extracellular receptor Angiotensin Converting Enzyme 2 (ACE2) and the serine protease transmembrane serine protease 2 (TMPRSS2). ACE2 and TMPRSS2 are expressed in both nasal and bronchial epithelium as detected by immunohistochemistry.[7] Single cell RNA-sequencing analysis confirmed that ACE2 is enriched in the human airway epithelium including club, ciliated and goblet cells, AT1 and AT2 cells.[8,9] ACE2 and TMPRSS2 are also co-expressed in a subpopulation of ciliated cells and AT2 cells.[8] Consistently, histology characterization revealed that SARS-CoV-2 infection induces severe damages in the intrapulmonary airways and alveoli.[10] However, detailed characterization of different respiratory cell types remains lacking for COVID-19 patients. In this report we showed that ciliated, club, AT1 and AT2 cells are the major cell types damaged by SARS-CoV-2 infection. More importantly, we demonstrated that distinct proliferating cells are present in the trachea/large airways, small airways and alveoli following SARS-CoV-2 infection.

We examined tracheas and lungs from five deceased patients with postmortem intervals (PMIs) as low as 2.5 hours. The epithelium was severely damaged in some parts of the trachea (Supplementary information, Fig. S1a, b). Ciliated and club cells were shed into the lumen, and the underlying basal cells were exposed (Fig. 1a, b and data not shown). Although KRT5 remained to be expressed in these exposed basal cells, the nuclei rounded up in contrast to the neighboring basal cells where the overlaying club and ciliated were intact (Supplementary information, Fig. S1c). Intriguingly, although basal cells and other epithelial cells rarely proliferate at homeostasis in the adults,[1] extensive basal cell proliferation was observed in the trachea, especially in the area where club and ciliated cells were damaged (Fig. 1b). The proliferating cells were limited to the immediate parabasal layer in the area where the epithelial integrity was relatively well maintained (Fig. 1a). However, in the severely damaged area, proliferating cells were occasionally observed in the basal layer which is lined by basal cells with round nuclei (Fig. 1b). Together these findings demonstrate that in the trachea basal cells proliferate, likely serving as progenitor cells to regenerate the damaged epithelium following SARS-CoV-2 infection.

We next examined the COVID-19 lungs. The intrapulmonary airways and alveoli were severely damaged with epithelial denudation and extensive intra-alveolar fibrinous exudates (Supplementary information, Fig. S2a). A significant amount of ciliated and club cells were sloughed and detached from basal cells in some areas (Supplementary information, Fig. S2b). In the large airways (diameter > 0.5 mm), 82% of Ki67$^+$ cells co-express KRT5 (Supplementary information, Fig. S3a), suggesting that basal cell remains to be the major proliferative cell population in response to viral challenge. By contrast, in the small airways (diameter < 0.5 mm) although 29.08% ± 2.93% of basal cells showed Ki67 expression, the majority of proliferating epithelial cells were KRT5$^-$ (4.10% ± 1.53% SCGB1A1$^+$ KRT5$^-$ and 66.82% ± 3.31% SCGB1A1$^-$ KRT5$^-$). Goblet cells (MUC5AC$^+$) and ciliated cells (FOXJ1$^+$) were not proliferative (Supplementary information, Fig. S3a and data not shown, $n = 5$). Although a previous study showed that approximately 1% to 2% of neuroendocrine cells are proliferative,[11] we did not observe any Ki67$^+$ neuroendocrine cells (Synaptophysin, SYP$^+$) (Supplementary information, Fig. S3b, 0/16 cells). While extensive CD45$^+$ cells were present in the mesenchyme, the inflammatory cells did not seem to infiltrate into the epithelial layer (Supplementary information, Fig. S3c). We did not observe proliferating AT1 cells in the alveoli (Supplementary information, Fig. S4). 3.99% ± 1.66% of AT2 cells exhibited Ki67 staining in the parenchyma where the alveoli were relatively

Received: 24 May 2020 Accepted: 20 June 2020
Published online: 30 June 2020

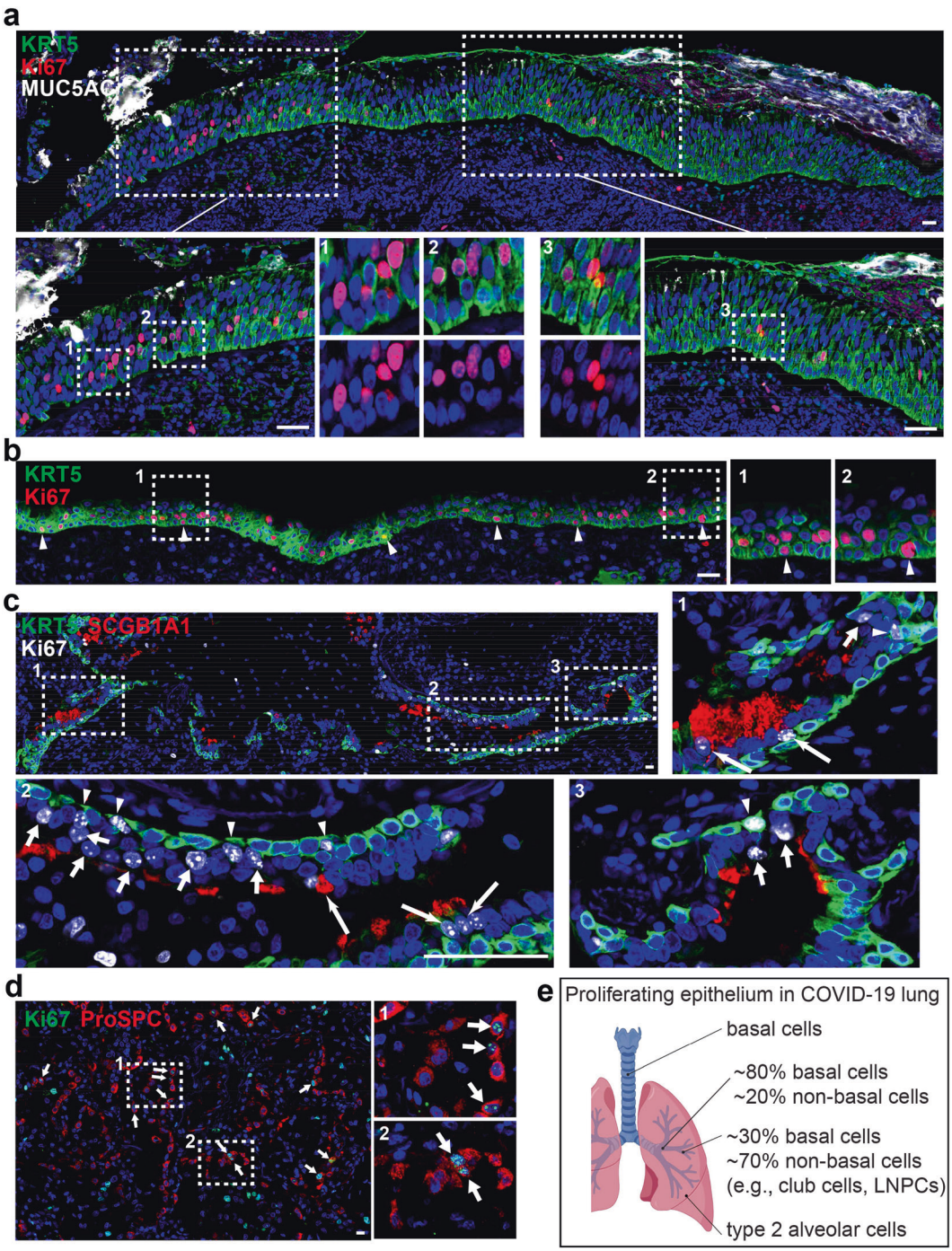

Fig. 1 Distinct proliferating cells in the trachea, intrapulmonary airways and alveoli of COVID-19 patients. a Proliferating basal cells are enriched in the area where severe damages occur. Note Ki67^{+} cells are limited to the immediate parabasal layer. **b** Proliferating basal cells are present in the basal (arrowheads) and immediate parabasal layers where the overlaying club and ciliated are depleted. **c** Proliferating cells in the intrapulmonary airways include basal cells (arrowheads), club cells (long arrows) and KRT5^{-} SCGB1A1^{-} population (arrows). **d** Extensive proliferating alveolar type 2 cells (arrows). **e** Schematics of proliferating epithelial cells in COVID-19 trachea and lung. Note the predominant proliferating cells in the small airways are Lineage Negative Proliferating Cells (LNPCs). In this study tracheas and lungs from five COVID-19 patients were examined. Scale bar, 50 μm.

well preserved. However, in severely damaged area 15.69% ± 1.87% of AT2 cells were proliferative (Fig. 1d; Supplementary information, Fig. S4), suggesting that AT2 cells are mobilized to regenerate the alveoli.

Taken together, we demonstrate that distinct cell populations proliferate in different regions of the respiratory system following SARS-CoV-2 infection (Fig. 1e). In the trachea and larger airways basal cells (KRT5^{+}) proliferate extensively. This is consistent with a previous report showing that approximately 84% proliferating cells express KRT5 in the normal large airways (>2 mm).[12] Interestingly, although approximately 30% proliferating cells exhibit KRT5 expression in the small airways of

COVID-19 lungs, the majority of proliferating cells do not express KRT5. Among them the predominant cell population does not express lineage markers including SCGB1A1, FOXJ1, acetylated tubulin, MUC5AC and SYP as evidenced by immunostaining. Whether these Lineage Negative Proliferating Cells (LNPCs) are similar to the LNEPs identified in mouse models[13] remains to be determined. It is also unknown whether these cells are derived from the underlying basal cells or neighboring club cells. The alveoli AT2 cells proliferate extensively in response to virus-induced damage. Mouse studies have shown that basal cells and AT2 cells consist of multiple subpopulations (reviewed by[6]). It will be interesting to further determine which subpopulations are activated and participate in lung regeneration in COVID-19 patients. Understanding the molecular mechanisms that promote the expansion of distinct progenitors present in the proximal/distal airways and alveoli is critical for rebuilding a functional respirator system.

ACKNOWLEDGEMENTS

We would like to thank Columbia University COVID-19 Hub and the autopsy pathologists who have been fighting in the front line and provided us low PMI specimens. We would also like to thank Dr. Hanina Hibshoosh at Columbia University Biobank and The Molecular Pathology Shared Resource for helping with tissue processing. This research is funded by HL132996 (J.Q).

AUTHOR CONTRIBUTIONS

Y.F. and J.Q. designed experiments, analyzed data and wrote the manuscript. Y.F. also performed the experiments. H.L and H.H prepared the paraffin and frozen sections. L.Q. revised the manuscript. H.L and A.S. performed pathological analysis.

ADDITIONAL INFORMATION

Supplementary information accompanies this paper at https://doi.org/10.1038/s41422-020-0367-9.

Competing interests: The authors declare no competing interests.

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