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SPOTLIGHT

Maintaining the barrier: New tactics to protect our breathing

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There is a significant gap between our mechanistic understanding of lung injury repair, thought to be a lengthy process, and observational studies which indicate it is extremely rapid. In this issue, Guild et al. (https://doi.org/10.1083/jcb.202212088) provide exciting new insights into the processes taking place during the first few hours following alveolar damage.

Every breath we take brings oxygen into our bodies and expels carbon dioxide. These gases are exchanged in the most distal parts of our lungs called alveoli, delicate epithelial structures consisting of just two cell types—thin and flat alveolar type I (AT1) cells, which are responsible for the exchange process, and cuboidal alveolar type II (AT2) cells. AT2 cells are responsible for the production of surfactant and also act as stem cells for the alveolar niche, with the capacity to both self-renew and differentiate into AT1 cells (1). Recent studies shed light on the multi-step repair process utilized by AT2 cells following lung injury, uncovering a discrete intermediate state achieved by these cells in response to tissue damage (2, 3). Given that injury affects both AT1 and AT2 cells, these processes require both proliferation of AT2 cells, to maintain the stem cell pool, and the differentiation of some of their progeny into AT1 cells. Although it has been possible to dissect this differentiation trajectory, the necessary sequence of steps results in a repair process that could last several days (4). The long-standing issue with this paradigm is that it would result in the alveolar barrier being compromised for an extended period of time following damage. This is contradicted by classical electron microscopy studies of lung injury, which show minimal denudation of the lung epithelium even after short periods of time (5, 6). In this issue, Guild and colleagues uncover a previously unappreciated mechanism underpinning the maintenance of lung barrier function following AT1 cell loss (7).

The authors set out to investigate the events surrounding the replacement of lost AT1 cells through an elegant combination of lineage tracing and targeted ablation of AT1 cells. Utilizing both genetic and chemical approaches to achieve this, Guild and colleagues were able to observe a complete restoration of the AT1 epithelial barrier as early as 2 h following injury, providing the first evidence showing the response of alveolar tissue in the short timeframe immediately following damage. Intriguingly, the newly generated AT1 cells did not bear the original AT1 lineage mark, indicating that they were not derived from the existing AT1 cells. Further investigation uncovered the involvement of the intermediate cell state, with additional AT2 lineage tracing confirming the new AT1 cells were derived from AT2 cells (2, 3). In an exciting twist, the cells were found to not be associated with proliferating AT2 cells, suggesting a direct conversion of AT2 cells into AT1 cells in a manner that uncouples the processes of proliferation and differentiation in the initiation of lung injury repair. This finding did, however, raise an important question—how is the AT2 cell pool maintained following this direct conversion?

The answer was found in a newly proposed subpopulation of AT2 cells uncovered through the analysis of proliferation kinetics immediately following injury. Guild and colleagues uncovered a significant proportion of proliferating AT2 cells containing nuclear doublets within 2 h of AT1 ablation. This timeframe was deemed too short to allow a cell to pass through all the required stages of the cell cycle, suggesting this subset of cells possessed 4n nuclear content at homeostasis. Subsequent analysis of this population also suggested it may possess stem cell features, characterized by active Wnt signaling (8, 9). Unlike mononucleated cells, binucleated AT2 cells do not enter either G0 quiescence or S phase. Instead, they rapidly divide into two daughter cells within 2 h of AT1 cell loss through a bona fide mitotic process. Therefore, the binucleated cells were postulated to enter the cell cycle directly into M phase, suggesting stable arrest in the G2 phase at homeostasis. This particular form of cell cycle suspension, while uncommon, has been previously described in other organisms with the capacity for rapid and robust regeneration (10). The presence of these cells in the lung enables the alveolar niche to fully commit part of its stem cell pool to the rapid restoration of the alveolar barrier, with binucleated cells subsequently replenishing AT2 cell reserves.

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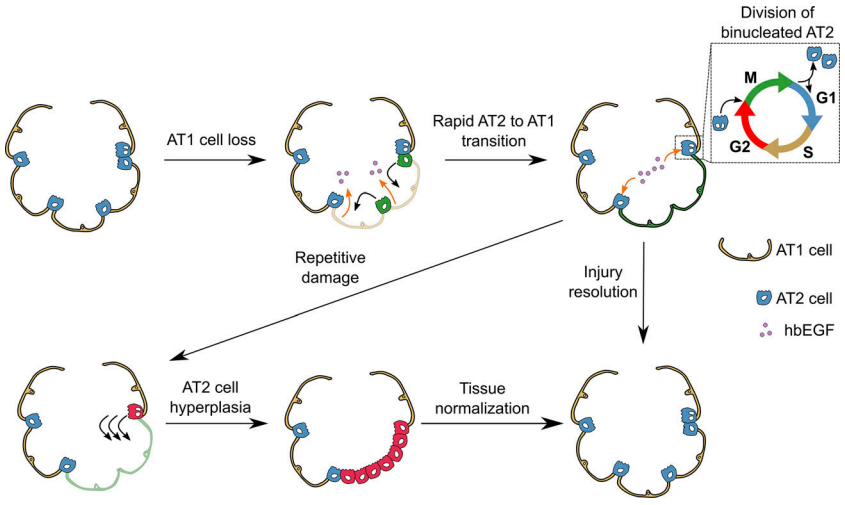

Figure 1. **AT2 cells preserve lung barrier function through rapid conversion and subsequent proliferation.** Upon loss of AT1 cells, AT2 cells rapidly transition to preserve the alveolar barrier. The release of hbEGF by dying AT1 cells activates the proliferation of AT2 cells, with binucleated AT2 cells entering into immediate mitosis prior to further proliferation to replenish the AT2 cell pool and resolve the injury. Repetitive rounds of damage lead to the development of a dysfunctional hyperplastic barrier that resolves into normal tissue architecture over time.

But which signals would trigger this replenishment, and which cells would provide them? Following confirmation of heparin-binding EGF-like growth factor (hbEGF) as the ligand required to drive post-injury AT2 cell proliferation, the authors postulated a mechanism whereby constitutively expressed hbEGF within AT1 cells is released as a result of damage-related protease activity. This provides the alveolar niche with a permanent fail-safe, where loss of AT1 cells immediately releases pre-existing proliferative signals for the AT2 cells, allowing for the lung repair process to commence as rapidly as possible.

Finally, Guild et al. (7) sought to understand how this reparative process would respond to multiple rounds of alveolar injury. Repeated AT1 cell ablation resulted in a hyperplastic phenotype comprised of cells expressing a mixture of AT1, AT2, and intermediate state characteristics, but lacking functional abilities such as surfactant production of efficient gas exchange. Interestingly, the hyperplastic alveolar tissue normalized itself to a homeostatic architecture within a month after injury. Although active Wnt signaling is required for this hyperplastic response, the mechanisms surrounding the subsequent normalization, including how cells are selected for either differentiation or clearance, and what drives each of these processes, remain unknown for now. Perhaps the surrounding tissue is capable of exerting some form of normalizing pressure on hyperplastic regions over time in the absence of damage-related signals?

Until now, observational studies of lung injury responses have been at odds with our understanding of how the integrity of the alveolar barrier is maintained by AT2 stem cells. In this elegant study, the authors have described a novel mechanism by which AT2 cells maintain the epithelial barrier by rapidly differentiating into AT1 cells immediately following injury (Fig. 1). The AT2 cell pool was subsequently replenished by rapid division and proliferation of a previously undescribed subpopulation of binucleated AT2 cells maintained in the G2 phase of the cell cycle, and thus primed for rapid mitosis. Guild and colleagues have also shown that this damage response is triggered by a fail-safe mechanism of constitutively expressed hbEGF within AT1 cells, which is released immediately upon injury. Finally, multiple rounds of AT1 cell loss drove the replenishing AT2 cells to transiently establish a hyperplastic, non-functional epithelial barrier that resolved into a normal architecture over time. Despite the apparently delicate and fragile nature of alveolar tissue, this study demonstrates not only its powerful abilities to adapt and respond to cell loss almost instantaneously, it also highlights the clear importance of lung barrier integrity, given the drive to preserve it above all other lung functions.

These new findings raised several outstanding questions. What signals trigger the initial rapid transition of AT2 to AT1 cells? Given a short timeframe, can any bulk AT2 population perceive the physical absence of AT1 cells and immediately flatten to cover the denuded alveoli? Do mononucleated and binucleated AT2 cells display different functionality? What factors dictate which AT2 cells will replenish the binucleated pool following injury resolution? What mechanisms govern the homeostatic G2 arrest of binucleated cells, and can they be used by other cell types in other tissues? Do other organs utilize similar rapid response mechanisms when injured? Guild and colleagues have provided a thrilling first step into understanding how lungs protect themselves in the first moments following damage.

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