Polyploidy in Lung Regeneration: Double Trouble or Dynamic Duo?

The alveolar epithelium consists of type 1 and type 2 alveolar epithelial cells (AECs), which form a tight barrier. During lung injury, AECs die, compromising barrier integrity. Barrier integrity can be restored via physiologic regeneration, yielding normal cell numbers, tissue architecture, and function, or by fibrotic repair, characterized by altered cellular makeup and scarring (1). During physiologic regeneration, surviving AEC2s proliferate and differentiate into AEC1s, restoring normal numbers of AEC2s/AEC1s. We and others recently identified a transitional cell state assumed by proliferating AEC2s as they exit the cell cycle (2–6); these cells enlarge and spread and express genes involved in protein synthesis, response to DNA damage, and cell cycle arrest. During physiologic regeneration, transitional AECs differentiate into AEC1s (5, 6). However, persistence of the transitional state is associated with pulmonary fibrosis (2–8). The mechanisms by which physiologic regeneration is diverted toward fibrotic repair are incompletely understood.

Polyploid cells contain more than two copies of the genome. They arise from endoreplication, in which cells replicate their DNA but do not divide (failed cytokinesis), or from cell–cell fusion (9). Polyploid cells are typically hypertrophic and can be binuclear or mononuclear, depending on whether the nucleus divides (karyokinesis) (Figure 1). Polyploidy is induced by cellular stress, such as DNA damage induced by exogenous insults or rapid clonal expansion. Therefore, polyploidy often arises as rapidly proliferating cells exit the cell cycle and enter a state of terminal differentiation or senescence (10). Polyploidy has been described in the liver, placenta, kidney, skin, brain, breast, and heart. We and others have described AEC2 hypertrophy in response to lung injury (11, 12). However, with rare exceptions (13), polyploidy has not been described in the alveolar epithelium.

In this issue of the *Journal*, Weng and colleagues (pp. 564–576) identify polyploid AEC2s during repair after lung injury (14). This novel and important finding was demonstrated in the bleomycin mouse model of fibrosis and in cultured AECs and by both immunostaining and flow cytometry. Elegant live imaging studies revealed failed cytokinesis as the mechanism. Polyploid AECs are hypertrophic, although not all hypertrophic AECs are polyploid. Although some polyploid AECs exist in the transitional AEC state, mature AEC2s and (at least *in vitro*) AEC1-like cells can also be polyploid. Sophisticated lineage-tracing studies using Confetti mice revealed mononuclear as well as binuclear polyploid AECs. Inhibition of the integrated stress response (ISR) prevented or attenuated AEC hypertrophy and polyploidy *in vivo* and *in vitro*. Taken together with prior work by these investigators demonstrating the pathogenic role of the ISR in fibrosis (15), these data suggest that ISR activation in proliferating AEC2s leads to cytokinesis failure, resulting in cell hypertrophy and polyploidy, which may lead to fibrosis. Thus, whereas physiologic alveolar regeneration restores normal cell numbers and tissue architecture via cell proliferation and differentiation, Weng and colleagues propose polyploidy, yielding altered cellular makeup, as a driver of fibrotic repair (14).

As with any study, minor technical limitations exist. Polyploidy was quantitated by planimetry, counting binucleated cells on two-dimensional sections. Because larger cells are more likely to be captured on two-dimensional sections (11, 16), the reported polyploidy rate may be an overestimate. *In vitro* data suggest but do not prove that the ISR drives polyploidy in a cell-autonomous fashion *in vivo*. However, that polyploid AECs arise during fibrotic repair after lung injury in an ISR-dependent manner is a novel and important finding that significantly advances our understanding of the mechanisms by which physiologic regeneration is diverted toward fibrosis, adds the lung to the list of mammalian tissues in which polyploidy arises after injury, proposes a novel mechanism by which the ISR drives fibrosis, and reaffirms the promise of ISR inhibition as a therapeutic strategy.

This pivotal study establishes a foundation for further investigation. First, the mechanism by which the ISR induces endoreplication should be studied. Mechanical tension and YAP/TAZ signaling have been implicated in failed cytokinesis (9) and in pulmonary fibrosis (7, 17); how cross-talk between YAP/TAZ, mechanical tension, and the ISR impairs cytokinesis should be investigated. Second, the mechanism by which AEC polyploidy might drive fibrogenesis remains to be determined. The authors reason that by generating fewer progenitor AEC2s than mitosis, endoreplication limits the future regenerative potential of the lung (14). However, endoreplication of cells at the leading edge of wounds can be synchronized with proliferation of cells distal to the wounds (10). Likewise, endoreplication of some AEC2s may be integrated with enhanced proliferation of other AEC2s or *Scgb1a1*$^{+}$ progenitors (18). In addition, under certain circumstances, polyploid cells can divide, restoring normal numbers of diploid cells (9). That ploidy reversal drives fibrosis resolution in the bleomycin model is an intriguing possibility. Finally, even if polyploid AECs have diminished regenerative capacity, exactly how a paucity of epithelial progenitors activates fibroblasts is unknown. Polyploid AECs in the transitional state likely activate fibroblasts via integrin $\alpha_v\beta_6$-mediated transforming growth factor-β activation (2, 3, 17).

More fundamental is the question of whether and under what circumstances polyploidy is pathologic versus adaptive. The ISR drives polyploidy (14) and drives fibrosis (15). However, whether the ISR drives fibrosis by driving polyploidy is not entirely clear. In other words, in the setting of lung injury and

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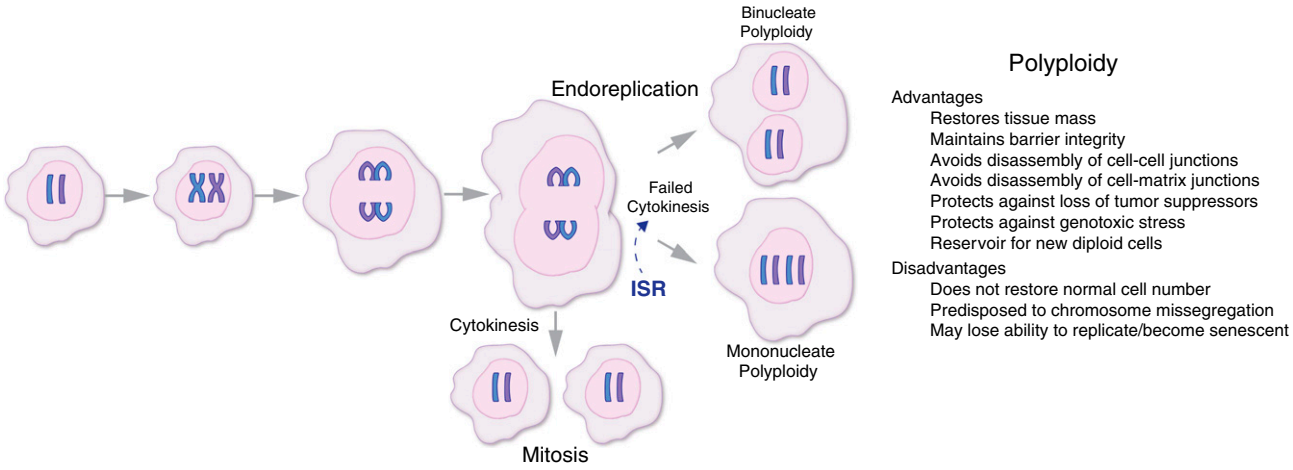

Figure 1. After duplication of the DNA during the S phase of the cell cycle, diploid cells can undergo cytokinesis, yielding two diploid cells (mitosis). During endoreplication, failed cytokinesis yields polyploid cells, which can be mononucleate or binucleate, depending on whether nuclear division (karyokinesis) is preserved. Polyploidy has advantages and disadvantages. In this study (14), the authors demonstrate that activation of the integrated stress response results in failed cytokinesis, leading to polyploidy. ISR = integrated stress response.

repair, is polyploidy pathologic or an adaptive response to cellular stress? Polyploidy offers several advantages over proliferation as a means to restore tissue mass after injury (Figure 1). Cell hypertrophy restores barrier integrity and tissue mass in a faster and more energy-efficient manner and avoids the cell–cell and cell–matrix adhesion disassembly that is required for cell division but compromises the barrier (10). Moreover, polyploidy arises in the setting of DNA damage, which can prevent cell proliferation. Moreover, polyploidy protects against cell death, the propagation of deleterious mutations after DNA damage, and loss of heterozygosity of tumor suppressor genes (9, 10). Finally, with an increased number of genomes, polyploid cells are capable of enhanced gene transcription and protein synthesis and are therefore inclined toward hypermetabolic states and cell growth (1, 9). An adaptive role for polyploidy in the context of regeneration has been demonstrated in the liver, kidney, and skin (1, 9, 10). However, polyploidy can also be maladaptive. When they do divide, polyploid cells are predisposed to chromosome missegregation (aneuploidy), driving oncogenesis (9, 10). Still, cellular heterogeneity due to aneuploidy may enhance organ-level fitness (9). Polyploidy impedes regeneration in some cell types, such as cardiomyocytes (1), although further polyploidization compensates modestly for cell death after injury (9).

The AEC transitional state is likely adaptive when transient but pathologic when persistent (2–7). The ISR evolved as an adaptive response to cellular stress, inhibiting global protein translation and promoting expression of molecular chaperones during impaired proteostasis. After lung injury, polyploidy likely initially arises as an adaptive response to cellular stress, promoting physiologic regeneration, but when persistently activated becomes pathologic, promoting fibrogenesis. Future studies aimed at dissecting the mechanisms by which adaptive processes such as the ISR, polyploidy, and the transitional state persist, are interdependent, and are hijacked to become pathologic may ultimately lead to the identification of novel therapies to promote physiologic regeneration and attenuate fibrosis. ■

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