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⊕ Selective Regulation of the Airway Mucin MUC5B in the Distal Airway

A common gain-of-function promoter polymorphism rs35705950 (G major allele, T minor allele) in the airway mucin MUC5B is the strongest and most replicated genetic risk factor for idiopathic pulmonary fibrosis (IPF) (1). It has been demonstrated that the MUC5B promoter variant affects MUC5B expression in the distal airways in IPF (2), and that Muc5b overexpression causes mucociliary dysfunction and enhances lung fibrosis in mice (3). However, less is known about the role this variant, located in a highly conserved region of the MUC5B promoter –3.5 kb upstream of the transcription start site (1), plays in transcriptional regulation of MUC5B. Publically available data through the Encyclopedia of DNA Elements (ENCODE) suggest this is a complex area of the genome with many transcription factors showing evidence of binding in the –3.5 kb region of the MUC5B promoter, in addition to the –0.1 kb proximal promoter (4). Another more general question in the field of mucin biology is that of selective mechanisms that differentially regulate MUC5B and its close neighbor and relative MUC5AC. Studies in recent years have shown that the transcription factors SPDEF (SAM pointed domain-containing ETS transcription factor) (5), NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells) (6, 7), and FOXA2 (Forkhead box protein A2) (4, 8) bind to both MUC5B and MUC5AC promoters, regulate their gene expression, and hence, lack the specificity needed to differentially regulate these two mucins (although FOXA3 is known to regulate MUC5AC specifically in Th2-dependent manner).

In this issue of the *Journal*, Chen and colleagues (pp. 220–234) identified a novel pathway that selectively regulates MUC5B, but not MUC5AC, expression in the distal airways (9). In their elegant and comprehensive report, they demonstrated three important

findings. First, the endoplasmic reticulum to nucleus signaling 2 (ERN2) selectively promotes expression of the MUC5B mucin in distal airways via its downstream effector, the spliced form of the XBP1S (X-box-binding protein 1) transcription factor. In a series of meticulous experiments, the authors used human IPF tissue, *in vivo* animal models, and primary cells to elucidate the role of the spliced form of XBP1 in regulation of MUC5B, but not MUC5AC, expression in response to stimulation with cytokine IL1 β . Among other findings, they show that there is a strong correlation of XBP1S and MUC5B mRNA on IL1 β treatment, but not at baseline, whereas correlation of MUC5AC and XBP1S is weak at baseline and after treatment with IL1 β . Second, XBP1S differentially regulates MUC5B promoter variant activity. Chen and colleagues report that induction of MUC5B(T) by XBP1S is greater than MUC5B(G) at all times tested by luciferase reporter activity. Finally, importantly, they also showed that pharmacologic inhibition and genetic deletion of ERN2-XBP1S reduced MUC5B expression. Inhibiting the ERN kinase had a moderate inhibitory effect, and deletion of XBP1 had a strong MUC5B inhibitory effect on expression levels. Higher levels of ERN2 and XBP1S were also observed in patients with IPF, and the results open potential avenues for novel therapeutic strategies using these observations. Using all data they collected, Chen and colleagues propose a “bistable model,” which is a positive feedback loop by ERN2-XBP1S that explains accumulation of mucus in IPF (Figure 1). This model exhibits both a reversible state (low stimulus) and an irreversible state (high stimulus). In response to insults that produce injury/or inflammation that accelerates MUC5B transcription, ER stress is induced, ERN2 is activated, and spliced XBP1 increases UPR gene and MUC5B transcription rates. This response is reversible on removal of the injury/cytokine stimulus. However, the presence of the MUC5B promoter minor allele amplifies XBP1S-induced MUC5B transcription, producing an irreversible positive feedback state that may be sufficient to trigger impaired host defense and accelerate cell senescence and/or damage.

The report by Chen and colleagues is a major step forward in understanding the selective regulation of MUC5B expression levels

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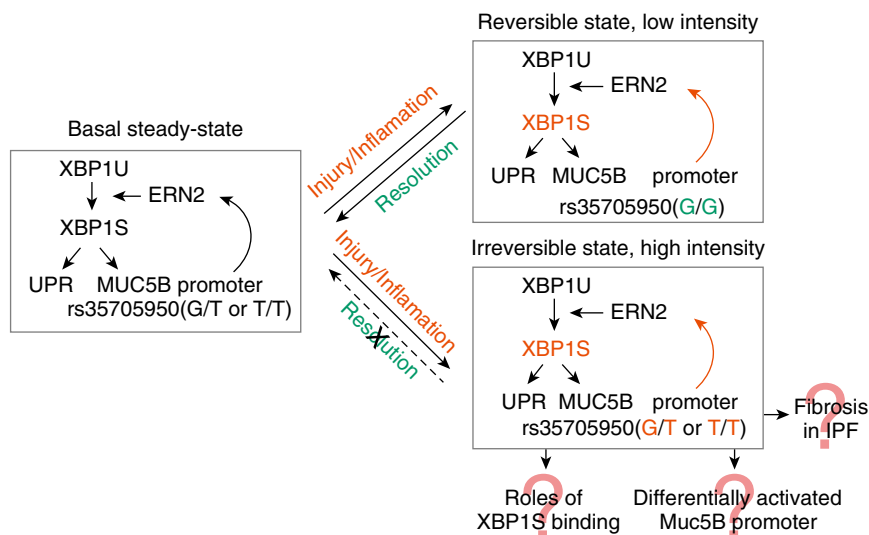


Figure 1. A bistable model of ERN2 (endoplasmic reticulum to nucleus signaling 2)/spliced form of XBP1S (X-box-binding protein 1)-mediated regulation of MUC5B and its promoter variant in distal airway epithelia of idiopathic pulmonary fibrosis (IPF). Adapted from Chen and colleagues (9).

in distal airways. The direct role of the spliced XBP1 on the MUC5B promoter, and especially the IPF-associated variant, further suggests that the described pathway is closely linked to disease pathogenesis. This is especially significant, as it highlights, for the first time, the role for ER stress in the airway epithelium in pathogenesis of IPF, in addition to previous reports in the alveolar epithelium (10). Overall, there is strong evidence presented for selective regulation of MUC5B, but not MUC5AC, as well as evidence for localization of the effect to the distal airway, which is of special importance in the context of IPF.

Naturally, the exciting results by Chen and colleagues raise many more questions. Although the evidence for selective regulation of MUC5B in the distal airways presented in the report is overwhelming, experimental evidence for the specific role of the rs35705950 variant is limited. In fact, the authors show that XBP1S binds predominantly to the proximal -0.1 kb promoter, and to a much lesser extent to the -3.5 kb region of the MUC5B promoter. Future work is needed to delineate respective roles of XBP1S and other transcription factors in binding this area of the promoter, as well as the effect of the variant on this binding. Furthermore, other regulatory mechanisms such as DNA methylation and histone modification will need to be taken into account to fully understand regulation of MUC5B in the distal airway. Another important area of future investigation will be understanding the role of XBP1S in pathogenesis of IPF by using animal and cell models of lung fibrosis. Importantly, the seminal study by Chen and colleagues opens up an entire line of investigation that should bring us closer to understanding regulation of MUC5B expression in IPF lung, fully elucidate the link of MUC5B overexpression and ER stress, and provide novel therapeutic options for this devastating disease with limited treatment options. ■

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Chasing the “Holy Grail”: Modulating Neutrophils in Inflammatory Lung Disease

Acute respiratory distress syndrome (ARDS) is a devastating condition characterized by severe hypoxemia, the accumulation of noncardiogenic pulmonary edema, and lung inflammation. ARDS affects >10% of all patients admitted to ICUs worldwide, and 35–45% of these patients die (1), predominantly of multiorgan failure. Survivors of ARDS are often left with significant long-term morbidities, and the healthcare costs associated with ARDS even 3–5 years after diagnosis match those of chronic conditions such as cardiac failure and chronic obstructive pulmonary disease (COPD) (2). Despite decades of research and many clinical trials, there remains no effective pharmacotherapy for ARDS.

Patients with neutropenia illustrate only too well the critical role of neutrophils in the defense of the host against invading microbes. However, although neutrophils are the “first responders” of the innate immune system, dysregulated neutrophilic inflammation occurs in a variety of acute and chronic lung conditions, including ARDS, COPD, bronchiectasis, and some subtypes of asthma (3). Neutrophils have long been recognized as key cells in the pathogenesis of ARDS, with clinical studies showing that neutrophil accumulation within the pulmonary vasculature occurs early in the evolution of the condition (4), and neutrophilic alveolitis is a histological hallmark (5). Neutrophilia is common in the BAL fluid of patients with ARDS (6), and the extent correlates with clinical outcome (7). Experiments using *ex vivo* neutrophils have shown marked alterations in the phenotype and function of cells obtained from the blood and alveolar compartments of patients with ARDS, including reduced rates of constitutive apoptosis (8, 9).

Neutrophils rely almost exclusively on anaerobic glycolysis for the generation of ATP and are therefore exquisitely adapted to undertake their functions in hypoxic environments (10). However, hypoxia has been shown to have marked effects on neutrophil function, including prolonging the lifespan (reducing apoptosis), enhancing degranulation, and impairing reactive oxygen species generation and oxidase-dependent killing of organisms such as *Staphylococcus aureus* (11). The effect of hypoxia on neutrophil survival has been shown to be mediated via stabilization of the transcription factor HIF-1 α (hypoxia-inducible factor-1 α), which

can itself be regulated via a family of enzymes that includes the PHDs (prolyl hydroxylase domain-containing enzymes).

The therapeutic “holy grail” for neutrophil-associated inflammatory lung disease is a treatment that can reduce the unwanted effects of neutrophils without compromising host defense. Building on their previous work demonstrating the critical role of PHD2 in human neutrophils (12), in this issue of the *Journal*, Harris and colleagues (pp. 235–246) show for the first time in both murine models and human neutrophils that IL-4 is able to ameliorate the delayed apoptosis of neutrophils resulting from exposure to hypoxic environments (13). The observed IL-4-induced effect on neutrophil apoptosis was mediated via STAT 3/6 (signal transducer and activator of transcription 3/6) signaling, and was dependent on the expression of PHD2, which downstream led to the degradation of HIF-1 α and increased rates of neutrophil apoptosis. Interestingly, IL-4 increased neutrophil apoptosis both under hypoxic conditions and after LPS challenge, without reducing the number of neutrophils initially recruited to sites of inflammation or compromising reactive oxygen species generation, raising the possibility that this may be an axis via which the delayed clearance of neutrophils observed in inflammatory disease may be targeted without impairing the host defense.

Of particular note, IL-4 was effective in modulating neutrophil lifespans even when administered after the onset of inflammation, supporting the concept that it may be suitable as a therapy for patients with established neutrophilic inflammation. To investigate whether the observations made in mice and purified human neutrophils were relevant to human disease, Harris and colleagues sampled BAL from patients with ARDS. They found that the lavage contained elevated concentrations of IL-4 compared with samples obtained from the lungs of healthy control subjects, and furthermore that circulating blood neutrophils from patients with ARDS exhibited increased expression of IL-4 receptor α .

Translating IL-4 into a therapy for inflammatory lung disease will require much more work, including exploring whether IL-4 supplementation can, in addition to modulating neutrophil lifespans, improve organ dysfunction and ultimately clinical outcomes. However, the incidence of neutrophil-associated inflammatory disease in which this therapeutic strategy may be of benefit is high and currently represents a pressing and unmet clinical need. The required initial steps along the translational path will likely include validating the current finding of elevated concentrations of IL-4 in the lungs of patients with inflammation, as well as demonstrating increased expression of the IL-4 receptor on neutrophils obtained from the same compartment—something that

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