The -Omic Approach to Understanding Glucocorticoid Effects in Smooth Muscle: Diving for Pearls

Corticosteroids represent a therapeutic mainstay for asthma in adults and children (1, 2). Corticosteroids bind and activate the glucocorticoid receptor (GR), influencing gene expression (3) and thereby alleviating inflammation and hyperresponsiveness. Although the anti-inflammatory effects of corticosteroids involve several cell types, including immune cells, a critical resident cell type is airway smooth muscle (ASM), which regulates airway tone and contractility. Furthermore, ASM is increasingly being recognized as playing immunomodulatory and synthetic roles that contribute to altered structure and function of asthmatic airways (4). In this regard, although corticosteroids are effective in limiting inflammation and AHR in many patients, airway remodeling, which involves increased proliferation and hypertrophy of epithelial cells and ASM as well as fibrosis, is not substantially alleviated (5). This major unmet clinical need is even more acute in the subset of severe asthma patients who are resistant or refractory to corticosteroids, experience more exacerbations, and have significantly more airway remodeling (6). Thus, there is an urgent need to understand the role of ASM in remodeling, and the mechanisms (if any) by which corticosteroids could blunt this progressive process. However, this requires elucidation of the signaling pathways in ASM that are activated by GR, and their interactions with proinflammatory and remodeling processes.

GR signaling and downstream effects are cell-type and context specific. They are influenced by previous drug exposures and patient-specific factors (e.g., age and sex), necessitating a “systems-biology” approach to gain a comprehensive understanding. Nonetheless, there is substantial value in “deep dives” that dissect GR mechanisms in a single cell type, in a single clinically relevant, unresolved context, i.e., remodeling. In this issue of the *Journal*, Sasse and colleagues (pp. 226–237) utilize a transcriptomic approach to understand how dexamethasone modulates signaling mechanisms that contribute to human ASM hypertrophy (7). Prior studies found that corticosteroids increase expression of Krüppel-like factor 15 (KLF15), a transcription factor with GR-binding sites (8, 9) that is involved in metabolic gene regulation. KLF15 can suppress ASM proliferation, and GR and KLF15 form feed-forward loops in airway epithelial cell gene regulation (9). Building upon these findings, the authors sought to understand how GR and KLF15 inhibit human ASM hypertrophy, and found that KLF15 is a direct transcriptional target of GR that represses ASM hypertrophy both directly and acting in concert with GR. Chromatin immunoprecipitation sequencing (ChIP-seq) analysis of GR occupancy in the context of KLF15 expression identified PLCD1 (an isoform of the phospholipase C enzyme family that regulates cytoskeletal structure) as both a KLF15-regulated gene and a novel repressor of ASM hypertrophy, where KLF-15 induces GR effects on PLCD1. PLCD1 overexpression was found to blunt TGF- β -induced ASM hypertrophy and α -smooth muscle actin expression. Additional genes with inducible GR occupancy and anti-inflammatory roles include *IRS2*, *APPL2*, *RAMP1*, and

MFGE8. Interestingly, baseline GR occupancy was found in loci for cytokine genes (*IL11* and *LIF*), which are paradoxically abrogated by corticosteroid exposure, suggesting noncanonical mechanisms for cytokine repression by GR per se.

By using elegant approaches to define GR interactions with chromatin on a genome-wide basis in human ASM, Sasse and colleagues provide pearls of insight into canonical and noncanonical GR signaling mechanisms in airway cells. In particular, they identify the need for GR-inducible, feed-forward synergistic mechanisms such as KLF15 that converge (at least) upon remodeling pathways. The study underlines the complexity of GR signaling in ASM and underscores the need for more “deep dives” to understand not only the end molecular targets of glucocorticoid effects but also the intervening pathway(s). Indeed, although GR modulation of inflammation via activation and repression of inflammatory genes is well recognized, recent studies reveal a more complex reality that is likely cell and context specific (10). For example, using multiple EM for motif elicitation-ChIP analysis, Sasse and colleagues found that GR occupies numerous sites that do not encode classic GR binding motif sequences, such as the AP-1 and FOX binding sequences (7), highlighting the existence of additional modes of gene regulation that are relevant to inflammation and remodeling in asthma.

As with many logistically and financially burdensome transcriptomic studies, there are limitations to the study by Sasse and colleagues (as recognized by the authors). Nevertheless, their findings give rise to several exciting topics for future studies. Reliance on adenoviral overexpression of KLF15 and PLCD1 will likely result in supraphysiological levels, with unpredictable off-target effects that could lead to over- or misinterpretation of their canonical roles. Future assessments of baseline expression would inform us regarding the matching of physiological levels. Here, the reliance on ASM cells from a single patient, with single steroid doses and durations, limits our understanding of the normal variabilities in GR signaling and/or roles of downstream signaling cascades. Heterogeneity among individuals is only made more complex by asthma endotypes, necessitating a comparison between ASM from nonasthmatic and asthmatic patients, particularly those who are resistant to corticosteroids. Such comparisons represent a critical next step in elucidating the clinical relevance of feed-forward pathways such as GR-KLF15-PLCD1 in airway remodeling and its abrogation. Given the disruption of GR signaling and the persistence of ASM inflammation and remodeling in severe asthmatics, it would also be exciting to determine how GR, RNAP2, and KLF15 occupancy changes in conditions that promote steroid resistance.

The role and mechanisms of PLCD1 per se in cytoskeletal remodeling and inhibition of hypertrophy remain unclear. Indeed, Sasse and colleagues suggest that PLCD1 is likely not the sole mechanism by which GR and/or KLF15 inhibit hypertrophy (7). Here, KLF15 and downstream targets are particularly appealing, given the role of KLF15 in epithelial remodeling (9). This may represent a relevant pathway for multiple cell types in asthma.

The focus on ASM hypertrophy, and previously on proliferation, is justified given their contribution to airway thickening in asthma. However, there is increasing recognition that ASM is both the source and target of growth factors, inflammatory mediators, and extracellular matrix components (4). Future work exploring feed-forward loops in GR signaling in the context of these parameters should emphasize both the role of ASM in remodeling and the effects of corticosteroids on this cell type. Extending the concepts of Sasse and colleagues to pediatric wheezing and asthma would also be important given that remodeling begins early in life, particularly in children with recurrent wheeze (11). Indeed, recent studies using developing ASM have shown that corticosteroids modulate inflammation differently in fetal ASM compared with adult ASM (12).

Overall, the novel work by Sasse and colleagues highlights the need for cutting-edge techniques and analyses, to “dive deeper” in trying to gain an understanding of gene expression and regulation in airway cells. Such studies will help us further unravel the complex interactions that modulate inflammation and remodeling in asthma, and identify novel therapeutic avenues for asthmatics who respond poorly to corticosteroids. ■

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Rodney Britt, Jr., Ph.D.
Department of Anesthesiology and Perioperative Medicine
Mayo Clinic
Rochester, Minnesota

Y. S. Prakash, M.D., Ph.D.
Department of Anesthesiology and Perioperative Medicine
Mayo Clinic
Rochester, Minnesota
 and

Department of Physiology and Biomedical Engineering
Mayo Clinic
Rochester, Minnesota

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