

Strengthening epithelial barriers through modulation of the histone code in allergic diseases—a novel approach for preventing the atopic march?



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Epigenetic changes have recently become subject of great interest not only to better understand the altered gene regulatory landscape in asthma, atopic dermatitis, and other allergic diseases but also for their potential for individualized patient management in allergic diseases.¹ DNA methylation, posttranslational histone modifications, and noncoding RNAs in concert with large multiprotein chromatin remodeling complex and organize the genome in a cell-type-specific “reading frame,” which is perturbed in disease. Among them, microRNAs, small noncoding RNA targeting the 3′ untranslated region of messenger RNAs, build up a complex gene regulatory network and are important regulators for balancing the differentiation of immune cell populations participating in allergic inflammation. A number of miRNAs have been found altered in the skin of individuals with atopic dermatitis including miRNAs commonly altered in allergic diseases such as miR-146a/b and miR-155, and also miRNAs involved in keratinocyte differentiation, proliferation, and apoptosis.

In this issue of the *Journal*, Liew et al² describe the downregulation of miR-335 in skin lesions of patients with atopic dermatitis and identify in multiple elegant experiments the complex regulatory network through which the loss of this miRNA perturbs the tightly controlled gene expression switches during terminal differentiation of the basal layer of the skin and contributes to the aberrant epidermal differentiation

(Fig 1). These defects lead to the loss of the epithelial barrier of the skin—a hallmark of atopic dermatitis. However, the translational importance of the findings might go beyond atopic dermatitis because the development of atopic dermatitis early in life predisposes children to subsequent development of food allergy, allergic rhinitis, and asthma—a phenomenon known as the “atopic march.” A defective skin barrier thereby functions as an entry route for the sensitization to other allergens and the future development of food and other allergies. Following the identification of the presence of class I histone deacetylases (HDACs) at the promoter of the miRNA, Liew et al provide evidence that Belinostat, a pan-HDAC inhibitor (that has received regulatory approval in 2014 for the treatment of relapsed or refractory peripheral T-cell lymphoma), can reverse this defect not only in cell line models but also in an *ex vivo* human skin culture model.

Very simplified, histone lysine acetyltransferases lead to a more open chromatin structure, whereas deacetylation of histone proteins catalyzed by HDACs leads to a more condensed chromatin structure. Acetylation and deacetylation of histone tails as well as other nonhistone proteins also has an important role on cellular inflammatory gene expression profiles. HDAC inhibitors constitute a heterogeneous group of molecules with diverse chemical structures including short-chain fatty acids such as butyrate, hydroxamic acids, aminoanilides, and cyclic peptides that target in most cases several members of the 4 different classes of HDACs in humans. They are intensively investigated as treatment or adjuvant treatment for a number of cancers and also have a long history of use in psychiatric diseases as mood stabilizers and antiepileptics. The action of HDAC inhibitors depends nonetheless on the cellular context, the expressed HDAC isoforms, and the presence of inflammatory cytokines.

The study by Liew et al is part of a number of recent studies demonstrating a beneficial effect of blocking HDACs in allergic and other inflammatory diseases. Nearly a decade ago, it was shown that the HDCA inhibitor trichostatin A improved atopic dermatitis in a mouse model, reducing notably the expression of T_H2, but not T_H1, cytokines. Furthermore, HDAC inhibitors have long been shown to upregulate tight junction proteins, reinforcing cell-cell adhesion, influencing cell polarity, and ultimately permeability and barrier function of epithelia. In cellular models of inflammatory bowel diseases, HDAC inhibitors improved barrier function and the transepithelial electric resistance under inflammatory conditions³ similar to what Liew et al show. Connecting both expression of T_H2 cytokines and epithelial barrier function, Wawrzyniak et al⁴ showed that HDAC activity was increased upon T_H2 cytokine release, especially IL-4 and IL-13, in primary human bronchial epithelial cells from patients with asthma. Treatment with the class I and II HDAC inhibitor

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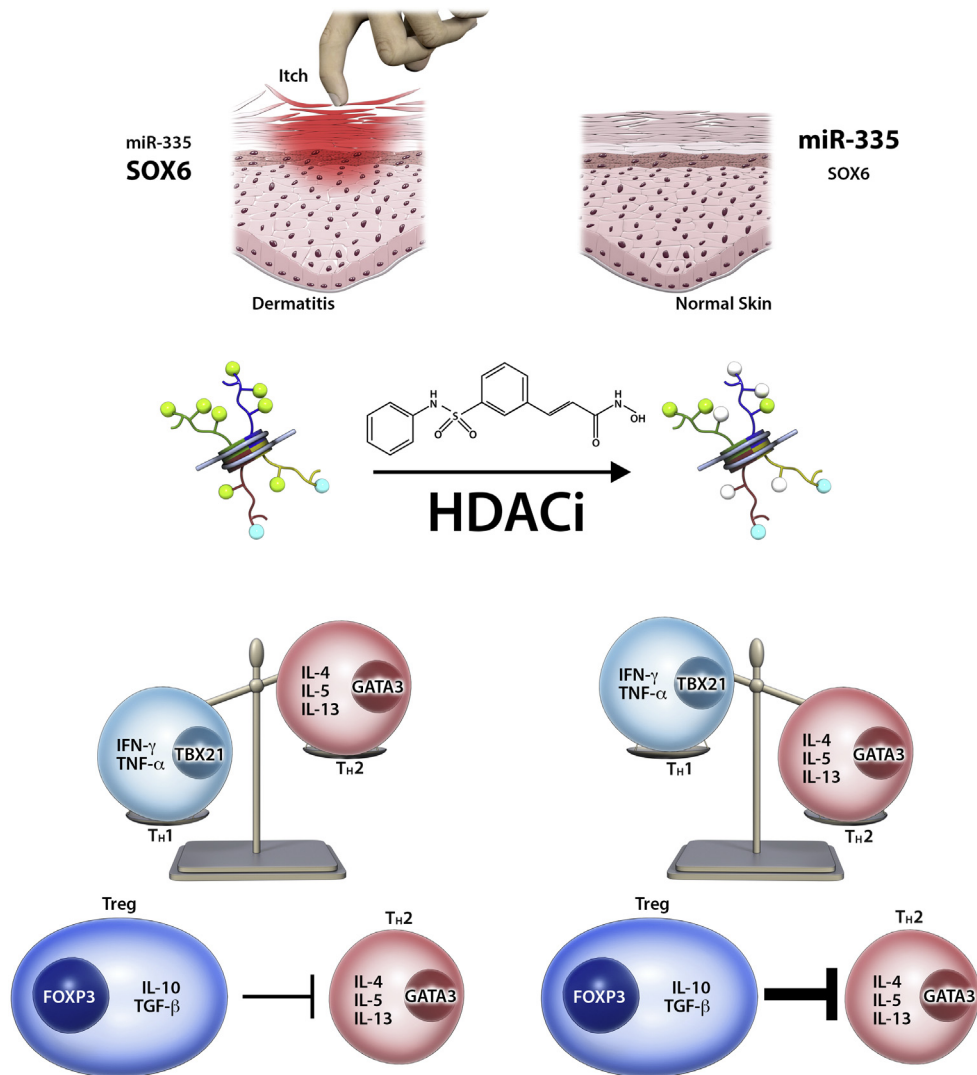


FIG 1. Simplified summary of the observed effects of HDAC inhibitors (chemical structure given for Belinostat) in allergic diseases including protection and repair of the epithelial barrier in multiple organs including skin, lung, and digestive tract, recalibration of immune cell populations favoring differentiation into T_H1 over T_H2 , and increased suppressive potential of Treg cells. Not all relevant cell populations are shown. *HDACi*, Histone deacetylase inhibitor; *Treg*, regulatory T cell. On the nucleosome, green spheres denote acetylated lysine residues, white spheres deacetylated lysine residues, and blue spheres methylated lysine residues. T_H1 cells are depicted in light blue, T_H2 cells in red, and Treg cells in dark blue.

quisinostat (JNJ-26481585) increased barrier function and rendered it less susceptible to barrier damage induced by the T_H2 cytokines.⁴ Furthermore, blocking of HDAC activity using the same drug was recently shown to restore the integrity of the nasal epithelium and mucosal function in patients with allergic rhinitis and prevented the development of airway inflammation and hyperresponsiveness in experimental models.⁵

Besides re-establishing barrier function, HDAC inhibitors have been shown to induce a number of processes that might be beneficial for the improvement of allergic diseases including restoring the balance of $CD4^+$ T-cell populations distorted in immune-related diseases,⁶ promoting the generation and suppressive function of regulatory T cells,⁷ and suppressing group 2 innate lymphoid cells (Fig 1).⁸ Nonetheless, it should be kept in mind that at least bronchial epithelial cells from patients with asthma have in general a hyperacetylated histone profile⁴ and

thus treating patients or cells further with inhibitors of deacetylation might induce an even larger divergence from the normal state.

Although the above-mentioned studies provide consistent evidence that increasing HDAC levels in allergic diseases might constitute a new way of treatment, the exact mechanisms of the anti-inflammatory response induced by HDAC inhibitors remain so far largely unelucidated. No detailed studies on genome-wide histone modification profiles before and after treatment with HDAC inhibitors are currently available to assess the effects at the genome-wide scale and to investigate whether the observed improvement in barrier function and other immunologic parameters is indeed due to changes in the histone code or other so far unknown effects. Our understanding of the mode of action of HDAC inhibitors is complicated by the fact that HDACs are ubiquitously expressed transcriptional corepressors that

target also a large number of nonhistone proteins and that are involved in a multitude of cellular pathways. Inhibition or modulation of these processes should be carefully investigated because it might provoke undesired adverse effects. Correct expression of HDACs is essential for proper function of the immune system including T-cell activation and differentiation as well as macrophage polarization.⁹ Furthermore, there have also been reports demonstrating a role for HDAC inhibitors in enhancing the expression of proinflammatory genes notably through modulation of the nuclear factor kappa B pathway, increased airway inflammation as well as abrogation of the innate immune response and downregulation of all essential functions of macrophages.

It should be emphasized that all studies showing efficacy of HDAC inhibitors in restoring epithelial barriers and rebalancing immune-cell populations have been performed in cell or organ culture or animal models. It remains so far unknown whether they would also show the same effect in the human body with its increased cellular diversity and complex interactions. It will be important to investigate also adverse effects of such a treatment because despite the large number of HDAC-inhibiting molecules identified and synthesized, most of them have an unacceptable toxicity profile for use in humans. Development of HDAC inhibitors targeting a specific class of HDACs or preferably specific members of a class has been a prerequisite for their clinical use in the field of cancer. Nonetheless, those in use still have a number of potentially serious side effects when given systematically including neurocognitive impairment, thrombocytopenia, and cardiac toxicity and are therefore given as therapy only for patients with late-stage cancer. This questions their potential use for a nonlethal disease such as atopic dermatitis. However, recently an HDAC inhibitor, Remetinostat, has been developed for topical applications and it is rapidly degraded upon release in the bloodstream because of the presence of metabolically labile groups. Remetinostat is in clinical trials for the treatment of cutaneous T-cell lymphoma and basal cell carcinoma; however, there are currently only intermittent results from 2 phase II trials in patients with basal cell carcinoma of suffering from mycosis fungoides available. Both studies confirm the low toxicity of the treatment, but it remains to be investigated whether such a treatment would have an effect in patients with atopic dermatitis.

There is substantial potential for the benefit of patients in the modulation of histone modifications in allergic diseases, not only by inducing histone deacetylation as shown by Liew et al, but there are also a number of molecules available for modulating other histone modifications such as histone lysine methylation. The Polycomb protein Ezh2, the main H3K27me3 methylase, has been shown to be critically involved in the differentiation and plasticity of CD4⁺ T_H1 and T_H2 cells and recent results show also

the possibility of improving allergic inflammation and airway hyperresponsiveness in experimental asthma models by administering an H3K27me3-specific histone demethylase inhibitor (GSK-J4).¹⁰

In summary, there is now sufficient evidence to support a potential beneficial role of modulating the level of HDACs in allergic diseases, which could in the future not only provide a novel form of therapy for the treatment of atopic dermatitis but may also prevent or slow down the atopic march and the development of future food and respiratory allergies. However, more ambitious and systematic investigation of the changes to the posttranslational modification profile of histone and other proteins as well as the other cellular and molecular parameters induced by the chemical modulation of the involved enzymes is required to better understand their mode of action and maintain the balance of the immune system. The possible synergistic or potentiating effects of combining HDCA inhibitors with other epigenetic modifiers such as GSK-J4 or modulators of bromodomain and extraterminal proteins will need to be explored in future studies, but they provide a new avenue for the treatment of patients with allergic diseases.

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