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FOXP3: Genetic and Epigenetic Implications for Autoimmunity

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Abstract

FOXP3 plays an essential role in the maintenance of self-tolerance and, thus, in preventing autoimmune diseases. Inactivating mutations of *FOXP3* cause immunodysregulation, polyendocrinopathy, and enteropathy, X-linked syndrome. FOXP3-expressing regulatory T cells attenuate autoimmunity as well as immunity against cancer and infection. More recent studies demonstrated that FOXP3 is an epithelial cell-intrinsic tumor suppressor for breast, prostate, ovary and other cancers. Corresponding to its broad function, FOXP3 regulates a broad spectrum of target genes. While it is now well established that FOXP3 binds to and regulates thousands of target genes in mouse and human genomes, the fundamental mechanisms of its broad impact on gene expression remain to be established. FOXP3 is known to both activate and repress target genes by epigenetically regulating histone modifications of target promoters. In this review, we first focus on germline mutations found in the *FOXP3* gene among IPEX patients, then outline possible molecular mechanisms by which FOXP3 epigenetically regulates its targets. Finally, we discuss clinical implications of the function of FOXP3 as an epigenetic modifier. Accumulating results reveal an intriguing functional convergence between FOXP3 and inhibitors of histone deacetylases. The essential epigenetic function of FOXP3 provides a foundation for experimental therapies against autoimmune diseases.

1. Introduction

FOXP3 is a member of winged helix/forkhead transcription factors and was identified because its mutations caused early onsets of fatal autoimmune diseases, which are now collectively called immunodysregulation, polyendocrinopathy, and enteropathy, X-linked (IPEX) syndrome, in mouse and human [1–5]. Independent studies by several laboratories have established that FOXP3 plays a critical role for the maintenance and function of regulatory T cells (Treg) [4, 6]. On the other hand, accumulating data have demonstrated that FOXP3 is disrupted in a broad-spectrum of cancer cells, including breast [7], prostate [8], ovarian [9], pancreatic [10], thyroid [11] and gastric [12] cancers, melanoma [13] and T-

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cell leukemia [14]. The disruptions confer growth benefit to cancer cells [7–9] as FOXP3 represses oncogenes [7, 8] while activating other tumor suppressor genes [15–17].

Since FOXP3 has emerged as an important tumor suppressor as well as an important immunological regulator [7, 8, 18, 19], therapeutic provision of FOXP3 to autoimmune patients and cancer cells has long been sought by immunologists and cancer biologists. Moreover, since the function of FOXP3 in both immune tolerance and tumor suppression depends on its transcriptional regulation, the mechanism of FOXP3-mediated gene regulation may hold the key to understanding both immune regulation and cancer biology as a potential gateway to translational medicine. Based on data from our laboratory [20] and those of others, we hypothesize that FOXP3 establish epigenetic landscapes to either repress or activate a broad spectrum of target genes. This concept provides a rationale for reconstituting FOXP3 function in cancer cells and autoimmune patients through modification of epigenetic machinery.

In this review, we will first focus on known genetic abnormalities of the FOXP3 gene in the IPEX syndrome, then discuss a molecular mechanism by which FOXP3 epigenetically regulates transcriptional activity of target genes. Finally, based on such an epigenetic mechanism regulated by FOXP3, we will explore clinical implications of histone deacetylase inhibitors (HDACi) for treatments against autoimmune diseases in animal models.

2. Genetic abnormalities of FOXP3 gene found in the IPEX syndrome

Germline *FOXP3* mutations are known to cause IPEX humans. As summarized in Table 1, a wide variety of mutations in the coding sequence of FOXP3 have been identified in the IPEX syndrome in humans [1, 3, 5, 21–59]. Moreover, substantial numbers of germline mutations in introns and polyadenylation sites of the *FOXP3* locus have also been reported (thoroughly reviewed in [60]). The most frequently affected mutations are found in the Forkhead domain of FOXP3 protein, although missense mutations are also found throughout the open-reading frame (Table 1). Since the conserved Forkhead domain possesses an essential role for the FOXP3 binding to its target gene promoters, mutations occurring in the Forkhead domain result in a substantial dysfunction of FOXP3 as a transcription factor in Treg cells. Mutations in the N-terminal domain of FOXP3 might disrupt its function as a transcriptional repressor, while mutations in the Leucin-zipper domain may abrogate protein-protein interactions of FOXP3 with its partner molecules.

Clinical symptoms caused by each FOXP3 mutation are also listed in Table 1 (and summarized in [60]). Specific correlations between FOXP3 genotype and clinical phenotype are unclear. Attempts to link the mutations to FOXP3 function in converting conventional T cells to regulatory T cells have largely been unsuccessful, as very little correlation was found between severity of IPEX diseases and the function of FOXP3 mutants in converting T cells to Treg. For instance, the R347H mutation, which causes lethal or severe IPEX in multiple patients, has very little impact in a battery of functional assays associated with regulatory T cells [40]. A more recent study showed that the function of Treg in the peripheral blood of 4 IPEX patients is unaffected by FOXP3 mutations [61]. Therefore, additional studies are needed to clarify the pathogenesis of IPEX patients. We have reported that FOXP3 mutations impact T cell functions by Treg-dependent and Treg-independent functions [62, 63].

As a transcription factor, FOXP3's function is reflected in both gene activation and repression. Linking the FOXP3 targets to Treg function depends on identifying the target genes whose up- or down-regulation may confer Treg function. However, this has proven exceedingly difficult, as Treg is manifested by cells that do not express it. As a result, the

molecular basis by which FOXP3 confers suppressor activity to Treg remains a mystery. Many of the technical difficulties may be related to the fact that Treg works *in trans* to suppress immune responses. In addition, if the relevant target genes are not identified, it is difficult to evaluate function of FOXP3 mutants. In contrast, FOXP3 is a cell-intrinsic tumor suppressor in cancer cells with clearly identified functional target genes [18, 19]. The relevance of mechanistic studies in cancer cells on the mechanism of autoimmune diseases is highlighted by substantial overlaps in FOXP3 targets between breast cancer cells and Treg. In addition, FOXP3 is also reported to be somatically mutated in various malignant tumors in humans [7, 8, 18]. The FOXP3 mutation spectrum found in human cancer cells is also enriched in the functional domains of FOXP3 such as N-terminal domain, zinc finger domain, leucin-rich domain and Forkhead domain [18]. In particular, it is worth noting that a mutation in the same position, P338, occurred in both IPEX patients and sporadic breast cancer samples [7, 60], although the somatic allele in cancer and germline allele in IPEX patients are distinct. Therefore, molecular characterization of FOXP3-mediated gene regulation in cancer cells may provide insights into Treg function.

A related question is whether germline mutations of FOXP3 increase the risk of cancer. Due to their low life span, t is unlikely to observe increased cancer incidences among IPEX patients. However, since female carriers of FOXP3 mutant alleles are asymptotic, it should be possible to carry out genetic epidemiology studies to determine the cancer risk of obligatory female carriers. In this context, *Foxp3*$^{sf/+}$ mice developed spontaneous mammary tumors and were more prone to DMBA-induced breast cancers, while prostate-specific deletion of *Foxp3* caused prostatic intraepithelial neoplasia in mice [7, 8].

3. Global analysis of direct FOXP3 targets in regulatory T cells and cancer cells

At least five global analyses of FOXP3 direct targets have been reported to date [20, 64–67]. Several important conclusions have emerged from these analyses. First, FOXP3 selectively binds to Forkhead motifs near the transcription start sites. In fact, our analysis revealed that FOXP3 mainly binds within 100 bp of the transcription start sites of its target genes [20]. Second, FOXP3 directly binds to thousands of genes in the genome and directly impacts their expression levels in hundreds, if not thousands, of genes. Since FOXP3 directly regulates many different aspects of T cell biology and cancer biology in different cell types, it is unlikely that the profound impact of FOXP3 on autoimmune diseases and cancer cells can simply be explained on the basis of only a few FOXP3 targets, nor should one expect that manipulations of a specific FOXP3 target, such as IL-35 [68], will allow cells to regain FOXP3 function. It is beneficial to study FOXP3 target pathways in a whole genomic manner in order to fully understand FOXP3 function in each cell lineage. Third, FOXP3 function is intricately intertwined with epigenetics. On the one hand, FOXP3-mediated gene regulation follows the histone code of gene activation and repression; by binding to gene promoters, FOXP3 dramatically changes histone modifications such as methylation and acetylation of specific histone tails [20, 65, 67, 69]. On the other hand, FOXP3 may regulate expression of the writers of histone code, i.e. the histone modification enzymes [16, 20, 67, 70].

Despite these common themes, there are major discrepancies as to the identity of FOXP3 targets in different studies [20, 64–67]. While it is premature to reconcile these differences, at least two factors must be considered. First, these analyses used either array analysis (ChIP-on-Chip) or next generation sequencing (ChIP-seq) to identify FOXP3 target genes. Second, different controls were used as FOXP3-negative cells. Three groups compared different T cells with or without high levels of endogenous FOXP3, while others engineered FOXP3 expression from the same cells in order to avoid comparing cells of fundamentally

different lineages. The challenge of lineage complexity [71, 72] is highlighted by the fact that the least concordance was observed between two studies that used a similar technological platform and a reference genome but used different controls of $FOXP3^{-}$ cells [65, 67].

4. FOXP3 establishes epigenetic landscapes by pull-push mechanisms

It is now generally accepted that FOXP3-mediated gene regulation depends on epigenetic mechanisms. For example, genes activated by FOXP3 have histone marks of H3K4 trimethylation and histone acetylation, while genes repressed by FOXP3 often have histone marks of H3K27 trimethylation [20, 67]. FOXP3 may therefore be an important epigenetic modifier in the regulations of target gene expression. At least two mechanisms may be envisaged for how FOXP3 regulates gene expression depending on epigenetic histone modification. First, FOXP3 may actively set the epigenetic landscape of target genes. In its simplest form, this model suggests that FOXP3 directly recruits or repels histone modification enzymes. For simplicity, we refer to this model as a “deterministic model.” Second, FOXP3 may explore pre-existing epigenetic landscapes of target genes. Since FOXP3 targets genes that have already been epigenetically programmed for its expression, it would be passive or parasitic in epigenetic regulation of gene expression. For simplicity, we refer to the latter as a “parasitic model.”

The parasitic model is based largely on a study using deep sequencing of DNaseI hypersensitive sites in $FOXP3^{+}$ and $FOXP3^{-}$ CD4 T cells [70]. This analysis identified more than 100,000 such sites in both subsets of cells. Since greater than 99% of the sites are overlapped in the two subsets, and since FOXP3 bound sites are within the overlapping gene set, the authors argued that FOXP3 does not alter epigenetic landscapes of their targets. For one to accept this argument, one must also accept that all of the $FOXP3^{-}$ CD4 T cells are the precursor of the $FOXP3^{+}$ cells. Since only a minute fraction of CD4 T cells are committed to express FOXP3 (as an immediate precursor of $FOXP3^{+}$ cells [71, 72]), the overwhelming majority of the $CD4^{-}$ cells used were not the precursors. As such, caution should be raised when considering adopting this parasitic model.

Since FOXP3 influences various histone modifications, including H3K27me3, H3K4me3 and acetylation of H3 and H4 at binding loci [20, 67, 69], it is plausible that FOXP3 works in concert with histone modification enzymes to exert its function as a transcription factor. Supporting this hypothesis, recent studies revealed that FOXP3 interacts with histone acetyltransferase TIP60, HDACs and histone modifying complex EOS/CtBP1 on chromatin to repress its target genes [69, 73]. In breast cancer cells, we reported that FOXP3 increases H3 acetylation by removing HDAC2 and HDAC4 from its binding site at the *p21(WAF1/CIP1)* locus [16]. While Foxp3 appears to increase H3K4me3 levels at its binding sites in T cells [67], the general mechanism of FOXP3-mediated gene regulation remains elusive.

In order to reveal the general mechanism of FOXP3-mediated gene expression, we first carried out chromatin-immunoprecipitation followed by ChIP-seq to identify FOXP3-binding sites in breast cancer cells. In combination with microarray analysis, we identified at least 845 direct targets of FOXP3 [20]. FOXP3-mediated gene activation correlated with both H4K16ac and H3K4me3. For multiple FOXP3 target genes, these crucial epigenetic modifications are simultaneously achieved by recruiting MOF and by displacing a H3K4 demethylase PLU-1. Our data suggest a pull-push model for gene activation by transcription factors. By pulling and pushing histone modification enzymes, a single transcription factor may provide necessary epigenetic codes for gene activation. These data provide definitive evidence for the deterministic model of epigenetic alterations of FOXP3 targets. Likewise,

FOXP3 may directly recruit or repel enzymes that write histone codes for gene repression. A putative model for FOXP3-mediated gene regulation is depicted in Figure 1.

5. Implications for autoimmune diseases

It is increasingly clear that environmental factors play major roles in the pathogenesis of autoimmune diseases. This major theme has been emphasized in several excellent recent reviews [74–80]. The conference also brought in a steady stream of exciting updates that substantially strengthen the notion that epigenetic modifications of both DNA and histone play an essential role in pathogenesis of autoimmune diseases. Disruptions of wide variety of epigenetic machineries have been implicated both in etiology and therapeutics of a number of autoimmune diseases in humans and in animal models: particularly in systemic lupus erythematous (SLE) and rheumatoid arthritis (RA). For example, drugs that inhibit DNA methyltransferase have been reported to be highly effective against SLE [81]. Moreover, several studies have shown that HDACi significantly ameliorate multiple experimental autoimmune disease models for SLE, RA, experimental autoimmune encephalomyelitis (EAE), inflammatory bowel disease, organ allograft models, and so forth [82–88] (summarized in Table 2). Importantly, HDACi have also been extensively investigated as anti-autoimmune therapeutics in clinical trials [89]. Among them, a recent clinical trial demonstrated that Givinostat, an inhibitor for class I and class II HDACs, achieved an efficacy of American College of Rheumatology Pediatric 70 for 67% of patients with juvenile idiopathic arthritis after 12 weeks of treatment [90]. In 2010, Givinostat was approved as an orphan drug for the treatment of juvenile idiopathic arthritis in Europe.

Given the important implications of FOXP3 in autoimmune diseases, it is of great interest to determine whether or not the HDAC inhibitors may work through FOXP3. Accumulating data from several laboratories has revealed that HDACi may promote FOXP3 function through three mechanisms (Fig. 2). First, FOXP3 has been shown to recruit histone acetyltransferase to the target genes. Our recent data show that FOXP3 interacts with and recruits MOF onto its target genes, regardless of whether the targets are activated or repressed by FOXP3 [20]. FOXP3-mediated gene activation is attenuated by MOF silencing and thus requires H4K16 acetylation [20]. Since H4K16 acetylation can be enhanced by HDACi, HDACi should phenocopy at least part of FOXP3 function. Likewise, FOXP3 causes gene activation by reducing HDAC2 and HDAC4 at its target genes such as p21 [16]. Second, FOXP3 directly inhibits enzymatic activity of HDAC1 to modulate gene expression in T cells [91]. One may conjecture that HDAC1 inhibitor partially substitutes for FOXP3 function in T cells. Third, FOXP3 acetylation TIP60 has been suggested to be critical for Treg function [73]. Such acetylation of FOXP3 protein may also be achieved by HDACi. Nevertheless, additional studies are needed to definitively link the therapeutic effects of HDAC inhibitors to the epigenetic mechanisms played by FOXP3.

6. Conclusions

Germline FOXP3 mutations are known to cause severe autoimmune diseases affecting multiple organs in humans. At the same time, somatic FOXP3 mutations in epithelial cells are reported to give rise to malignant tumors in mice models [7, 8, 18]. Therefore FOXP3 holds the key to molecular mechanisms of both autoimmune diseases and cancer. FOXP3 is known to play an important role as a transcriptional activator/repressor for its target genes in both Treg cells and epithelial cells. However, the molecular mechanism of the FOXP3-mediated gene regulation has not been fully characterized. The association between FOXP3-mediated gene regulation and histone codes for gene activation and repression is well established. While we and others have proposed that FOXP3 interacts with a number of histone modification enzymes to exert its function as an epigenetic modifier for its target

gene promoters [20, 69, 73], one group has suggested that FOXP3 plays no active role in establishing epigenetic landscapes of the target genes [70]. Given the critical role of FOXP3 in both autoimmune diseases and cancers, therapeutic targeting of FOXP3-mediated epigenetic machineries represents a novel approach to reconstitute FOXP3 function. Apart from the approved use of Givinostat for juvenile arthritis in Europe, several lines of evidence obtained from animal models of autoimmune diseases imply that a variety of HDACi maybe promising therapeutic agents for a wide variety of autoimmune diseases (Table 2). It remains to be established to what extent the therapeutic effects are attributable to reconstitution of FOXP3 function. Further understanding of the molecular mechanisms of FOXP3 in both Treg and cancer cells will not only aid to establish the significance of FOXP3 in the therapeutic effects of HDACi, but also promote development of selective and effective FOXP3-restoring drugs in autoimmune diseases.

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Research highlight

Multiple *FOXP3* mutant alleles have been identified in IPEX patients

Not all FOXP3 mutations abrogate regulatory T cell function

FOXP3 sets epigenetic landscape of its target genes

FOXP3 defects maybe corrected through targeting epigenetic machineries

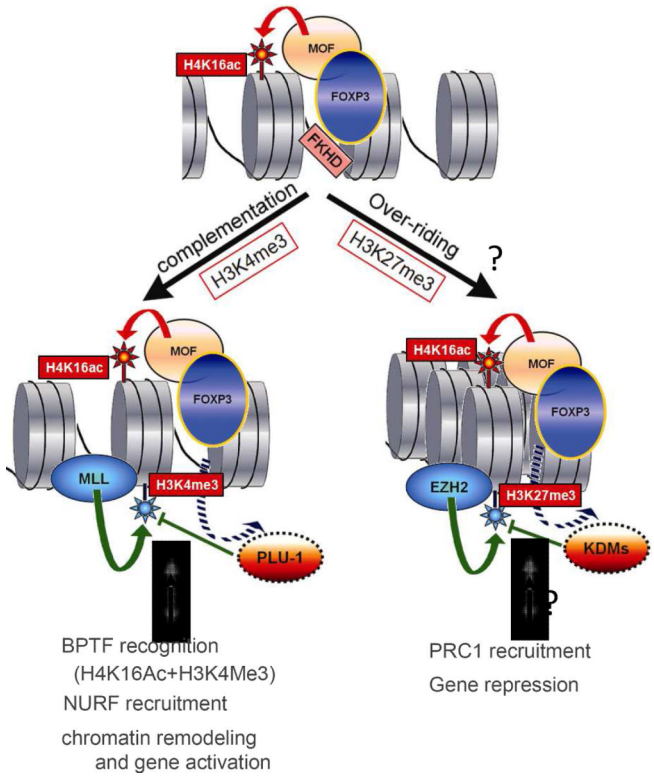

Figure. 1.

A pull-push model for FOXP3-mediated gene regulation. According to this model, FOXP3 recruits MOF to its binding sites, resulting in H4K16 acetylation (H4K16ac) of target genes. The consequences of this modification can be determined by subsequent tri-methylation (me3) at either H3K4 or H3K27. Gene activation can be achieved if H4K16ac is complemented by H3K4me3. This might be achieved by displacements of histone trimethyl-de-methylase, such as PLU-1, from its binding sites. This H4K16ac/H3K4me3 histone code will be recognized by Bromo- and PhD-domain containing BPTF which recruits Nurf complex to the loci. On the other hand, we speculate that the potentially active H4K16ac can be overridden by H3K27me3. The latter may possibly be achieved by either recruitments of EZH2 or displacements of H3K27me demethylase KDM6A or KDM6B. The repressive H3K27 code will be recognized by PRC1 which in turn recruits HDAC for gene repression.

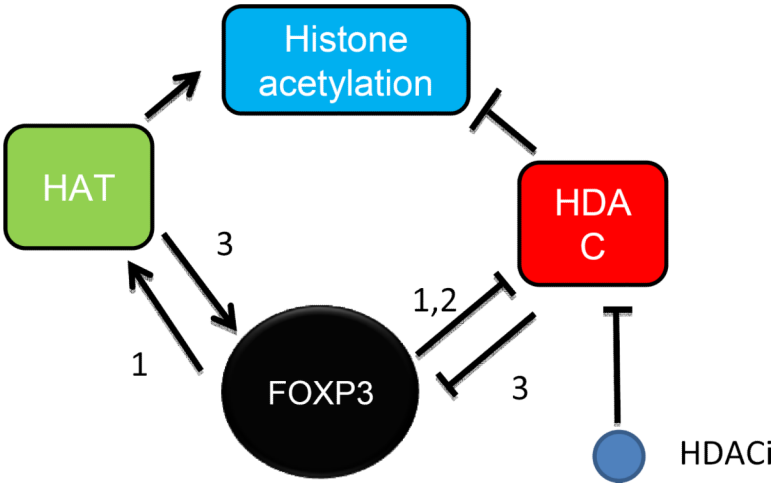

Figure. 2.

Functional convergence between FOXP3 and HDAC inhibitors (HDACi). 1: FOXP3 promotes histone acetylation by either recruiting MOF to, or displacing HDACs from, FOXP3 target genes, while HDACi achieves the same goal by reducing histone deacetylation. 2: FOXP3 and HDACi both inhibit HDAC activity. 3: Since FOXP3 activity is regulated by its acetylation by TIP60, a general HDACi may have a similar effect on FOXP3 activity.

Table 1

Germ-line mutations of FOXP3 gene found in the IPEX syndrome.

Domain	Genomic Alteration	Amino Acid Alteration	Examples of Clinical Symptoms	Reference
N-terminal domain	2C>T	M1T	diarrhea and/or sepsis, etc	29
	3G>A	M1I	diarrhea, hypothyroidism, lymphadenopathy and/or hepatosplenomegaly, etc	22, 29, 40, 47
	210G>T	Q70H	diarrhea, eczema, infection, autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura, anemia, and/or hypogammaglobulinemia, etc	33, 58
	227delT	L76 frameshift	diarrhea, thyroiditis, autoimmune hemolytic anemia, neutropenia, thrombocytopenia, infection and/or hepatitis etc	28, 35, 36, 45, 46, 52
	303_304delTT	F102 frameshift	diarrhea, alopecia, autoimmune hemolytic anemia, lymphadenopathy, hypothyroidism, membranous glomerulonephritis, food allergy and/or infections, etc	42, 50
	323C>T	T108M	diarrhea, pneumonia, pericarditis and/or arthritis, etc	25
	560C>T	P187L	diarrhea, dermatitis, cheilitis, onychondystrophy, infection, autoimmune thrombocytopenia, food allergy and/or basedow disease, etc	31, 41, 48
Leucin-zipper domain	725T>C	L242P	diarrhea, eczema, sepsis and/or nephropathy, etc	29, 47
	748_750delAAG, 543C>T	K250del	diarrhea, arthritis, idiopathic thrombocytopenic purpura, hepatomegaly, hepatitis and/or renal insufficiency, etc	5
	748_750delAAG	K250del	diarrhea, eczema, food allergy, nephrotic syndrome, infection, autoimmune hemolytic anemia, sepsis and/or autoimmune hepatitis, etc	32, 38, 45
	750_752delGGA, 1044+4A>G	splicing abnormality, E251del	diarrhea, autoimmune cytopenia and/or food allergy, etc	3
	751_753delGAG	E251del	diarrhea, eczema, thyroiditis, autoimmune hemolytic anemia, infection, sepsis, hypothyroidism, nephritis and/or autoimmune hepatitis, etc	30, 31, 41
	817G>T	A273S	diarrhea, eczema and/or thyroiditis, etc	57
	970T>C	F324L	diarrhea and/or pneumonia, etc	21
	970T>C, 543C>T	F324L	diarrhea and/or asthma, etc	22, 29, 40, 47
Forkhead domain	1010G>A	R337Q	diarrhea, etc	52
	1015C>G	P339A	diarrhea, eczema, autoimmune hepatitis, autoimmune hemolytic anemia,	29, 41, 47, 52

Domain	Genomic Alteration	Amino Acid Alteration	Examples of Clinical Symptoms	Reference
			hepatosplenomegaly and/or thyroiditis, etc	
	1037T>C	I346T	diarrhea, infection and/or sepsis, etc	47
	1040G>A	R347H	diarrhea, infection, sepsis, autoimmune gastritis, thrombocytopenia and/or infection, etc	5, 40, 53
	1040G>T	R347H	diarrhea, eczema, autoimmune hepatitis, autoimmune thrombocytopenia, anemia and/or food allergy, etc	29, 40, 47
	1061delC	P354Q frameshift	diarrhea, eczema, infection and/or dermatitis, etc	33, 58
	1080_1081insA	N361K frameshift	diarrhea, eczema, proteinuria and/or sepsis, etc	21
	1087A>G	I363V	diarrhea, thyroiditis, infections and/or sepsis, etc	35, 36
	1099T>C	F367L	diarrhea, eczema, liver dysfunction, thrombocytopenia and/or sepsis, etc	54
	1100T>G	F367C	diarrhea and/or tubulointerstitial nephritis, etc	48
	1101C>G	F367L	infection and/or sepsis, etc	31
	1110G>A	M370I	diarrhea, eczema, nephrotic syndrome, lymphadenopathy, splenomegaly and/or pneumonia, etc	21
	1113T>G	F371C	diarrhea, idiopathic thrombocytopenic purpura, autoimmune hemolytic anemia, autoimmune neutropenia, cholestatic hepatitis, autoimmune thrombocytopenia and/or infection, etc	5, 23, 31, 48
	1117T>G	F373V	diarrhea, etc	28, 36, 45, 56
	1117_1118TT>GC	F373A	diarrhea, etc	22, 29, 40, 47
	1121T>G	P374C	diarrhea, eczema, alopecia, autoimmune hemolytic anemia, autoimmune thrombocytopenia, membranous glomerulonephritis, sepsis, allergy and/or infection, etc	29, 31, 41, 48
	1139C>T	T380I	diarrhea, etc	59
	1150G>A	A384T	diarrhea, eczema, infections, hypothyroidism, thrombocytopenia, hypogammaglobulinemia, sepsis, alopecia, autoimmune neutropenia, thyroiditis, autoimmune hemolytic anemia, pancytopenia, asthma and/or pneumonia, etc	1, 5, 26, 27, 28, 29, 34, 39, 44, 45, 47, 51, 55, 57
	1157G>A	R386H	diarrhea, etc	57

Domain	Genomic Alteration	Amino Acid Alteration	Examples of Clinical Symptoms	Reference
	1169G>A	S390N	diarrhea, eczema and/or infections, etc	43, 57
	1189C>T	A397W	diarrhea, hypotonia, hypotheyroidism, therombocytopenia, peritonitis, cholangitis, cachexia, infections and/or sepsis, etc	5, 37
	1190G>A	A397Q	diarrhea and/or eczema, etc	57
	1222G>A	V408M	diarrhea, nephrotic syndrome, thyroiditis and/or infection, etc	52
	1226A>G	D409G	diarrhea and/or autoimmune hemolytic anemia, etc	50
	1271G>A	C424Y	diarrhea, eczema, food allergy, autoimmune hemolytic anemia, membranous glomerulonephritis and/or infection, etc	24, 50
	del1290_1309/insTGG	G430 frameshift	diarrhea, anemia, lymphadenopathy and/or sepsis, etc	5, 49
	1293_1294delCT	ter432 frameshift	diarrhea, etc	1, 36

Table 2

HDAC inhibitors and autoimmune disease models.

disease	animal	drug	prognosis	ref
experimental autoimmune encephalomyelitis (EAE)	C57BL/6 mouse	trichostatin A (TSA)	ameliorated EAE scores, reduced pro-Th1 and pro-proliferative mRNA in splenocytes	77
systemic lupus erythematosus (SLE)	NZB/W mouse	trichostatin A (TSA)	decreased anti-dsDNA antibody, increased Treg, decreased IgG and C3 deposits in kidney, decreased pathological glomerular diseases	76
autoimmune lymphoproliferative syndrome (ALPS)	MRL/LpJ-Tnfrsf6[lpr] mouse	valproic acid (VPA)	reduced lymphoproliferation, reduced weights and cellularities of spleen and lymph nodes, decreased double-negative T cells in spleen, lymph node and peripheral blood, increased histone acetylation in splenocytes	79
collagen-induced arthritis (RA)	DBA/1J mouse, Dark Agouti rat	MS-275	dramatic anti-rheumatic activity, prevented bone erosion and bone resorption, delayed onsets of arthritis, decreased serum IL-6 and IL-1beta	74
	DBA/1J mouse, Dark Agouti rat	suberoylanilide hydroxamic acid (SAHA)	moderate or slight reductions of arthritis, did not inhibit onset of arthritis	
collagen-induced arthritis (RA)	DBA/1 mouse	valproic acid (VPA)	decreased incidences and severities, increased both numbers and functions of Treg, decreased joint inflammation, decreased joint damage and destruction	78
collagen-induced rheumatoid arthritis (RA)	DBA/1 mouse	trichostatin A (TSA)	potently suppressed the severity of arthritis, suppressed T cell responses to type II collagen, increased production of IL-4, inhibited IFN-gamma expression	75
DSS-induce colitis	C57BL/6 mouse	trichostatin A (TSA)	substantially decreased colitis	80
	Hdac9-KO mouse	N/A	less colitis compared to wild type mice	
cardiac and islet allografts	BALB/c (donar) to C57BL/6 (recipient) mice	trichostatin A (TSA) with rapamycin	induced permanent, Treg-dependent allograft survival, induced donor-specific allograft tolerance	