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Chromatin remodeling complex in Treg function

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Abstract

Regulatory T cells (Treg), formerly known as suppressor T cells, are essential for maintaining self-tolerance as well as immune homeostasis. Lack of Treg or normal function of Treg often leads to lymphoproliferative syndrome and autoimmunity in human and mouse. The chromatin remodeling BAF complex regulates gene expression through the activity of Brg. Genetic ablation of *Brg* gene in mouse resulted in early embryonic lethality. T cell failed to develop in the thymus when *Brg* is deleted at DN stage. Using a *Brg* conditional KO mouse model, we deleted *Brg* at the DP stage in the thymus. Unexpectedly, T cells developed and matured normally. However, these mice displayed lympho-proliferative syndrome 2–4 months of age with enlarged peripheral lymphoid organs and leukocyte infiltration in non-lymphoid organs. T cells from these mice turned into effector cells producing increased amounts of effector cytokines as early as 4 weeks after birth. Further analysis revealed that the Treg population was specifically affected by *Brg* deletion. In this mini-review, we will discuss in detail the properties of Tregs controlled by Brg and the potential underlying mechanisms for an unanticipated, specific role of the Brg-containing BAF complex in controlling Treg functions.

Treg, its function and regulation

In 1995, a subset of T cells that actively suppressed immune responses were identified [1]. These cells, later referred to as regulatory T cells (Treg), are critical for maintaining self-tolerance and controlling immune responses. Defective Treg function invariably leads to autoimmunity or inflammatory diseases in human and mouse [2–5].

Based on the expression profiles of cell surface molecules and/or cytokines, several types of Tregs have been categorized [2]. Among them, naturally occurring Treg (nTreg) is the best characterized and studied. nTreg is a subset of CD4 T cells that develop in the thymus and constitutively express high levels of IL-2 receptor α chain (CD25) on the cell surface. nTregs comprise approximately 10% of peripheral CD4 T cells in mice and humans. In the absence of functional nTregs, systemic autoimmune syndrome develops in IPEX patients and Scurfy mice, underscoring the critical function of nTreg in self-tolerance [3,4]. A set of cell surface molecules besides CD25, such as cytotoxic T lymphocyte antigen-4 (CTLA-4), glucocorticoid-induced tumor necrosis factor receptor family related gene (GITR) and lymphocyte activation

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antigen-3 (LAG-3) have also been used to differentiate nTreg from conventional T cells [2]. TGF- β and IL-10, potent immune suppressive cytokines, are highly expressed in Tregs [6–10]. Although these genes are constitutively expressed at high levels in nTreg, they are also highly expressed in conventional T cells under certain conditions. In addition, although these genes contribute to the function of Tregs, none of them appears to be specifically involved in the generation and function of Treg cells. It was not until early 2000 that Foxp3, an X-linked transcription factor belonging to the fork-head family, was found to be specifically and highly expressed in nTregs [11–15]. More importantly, single gene inactivation of Foxp3 accounts for the loss of functional Treg cells and the development of IPEX in human patients and the Scurfy phenotype in mice [11,12]. In addition, forced expression of Foxp3 in non-Treg T cells endows suppressive activities [16,17]. Thus, a high level of Foxp3 now serves as a surrogate marker for functional Treg cells. Multiple reporter mouse strains have been developed to mark Foxp3 expressing cells [18–20], fueling our intense efforts of understanding how Treg function is controlled.

The existence of adequate numbers of functional Treg cells is essential for self-tolerance and immune homeostasis. Thus, perturbation of the generation, maintenance and the function of Tregs often lead to over-exuberant immune responses and immune pathology. High level expression of CTLA-4 and IL-10 contribute to the suppressive function of Treg without affecting its generation or maintenance [21]. On the other hand, IL-2 and its signaling components promote and PDE3B inhibits the peripheral maintenance but not the function of Treg cells [22,23]. TGF- β however regulates both the function and maintenance of Treg cells [24]. Therefore, it appears that multiple genes and signaling pathways are involved in the regulation of Treg maintenance as well as function. Substantial efforts are being devoted to unravel the mechanisms on how different aspects of Treg physiology are regulated. The hunt for genes that specifically regulate Treg function is warranted. Such studies are not only essential for the understanding of Treg function, but also for elucidating the etiology of human immune diseases, such as autoimmunity and cancer, and for devising effective therapies to treat these illnesses.

Chromatin remodeling and its function in T cells

In eukaryotes, genomic DNA associates with the nuclear protein complex known as chromatin. The basic repeating unit of chromatin is the “nucleosome core particle” where a histone octamer, consisting of 8 histone molecules of 4 types (two each of H2A, H2B, H3 and H4), grips and sequesters a 146-bp DNA segment. An array of linked nucleosome particles can be progressively coiled up into condensed fibers, effectively “burying” DNA. Such a structure on one hand protects DNA from harmful exposure to the myriads of proteins and enzymes in the nucleus. On the other hand, it prevents nuclear factors to access genes to regulate their expression.

To reconfigure chromatin, cells often mobilize “chromatin remodeling complexes” (CRC) that use energy to disrupt histone-DNA contacts. CRC are powerful molecular motors that use mechanical force derived from ATP hydrolysis to disrupt histone-DNA contacts, exposing nucleosomal DNA. Multiple classes of CRC have been isolated in eukaryotic cells, each of which contains a related ATPase as the motor subunit. The best-defined mammalian CRC is the “BAF complex”, a 10 subunit complex. Two ATPase subunits, Brg (Brahma-related gene) and Brm (Brahma) are found in mammalian cells to assemble into alternative BAF complexes [25,26] (hence the name Brg/Brm Associated Factor or BAF complex) [27]. The expression patterns of Brg and Brm are distinct: Brg is constitutively and ubiquitously expressed throughout development, while Brm is expressed predominantly in mature, differentiated cells [28,29]. The function of Brg and Brm are similar and yet divergent [28–32]. To regulate gene expression, CRCs often associates with Histone Acetyl Transferases (HATs) [33], histone

methylase [34], or Histone Deacetylases (HDAC) [35–37]. BAF complexes can be transiently recruited to and remodel a promoter to mediate gene induction in response to signaling, but can also constitutively remodel a promoter to poise it for rapid induction in anticipation of signaling.

Over the past few years, genetic and biochemical approaches have been used to address the roles of BAF complex in the immune system. T cell-specific inactivation of Brg containing BAF complex indicates that they are essential for thymocyte developmental transitions, and that they contribute to CD4/CD8 lineage bifurcation by directly repressing CD4 while activating CD8 expression [29,32,38]. A role of Brg in VDJ recombination has been proposed based on the fact that Brg stimulates recombination on chromatin templates *in vitro*, and that Brg is selectively bound to the TCR loci open for recombination [39–43]. Brg is also critical for innate and adaptive immune responses [44–46]. Thus, in the immune system, BAF complex possesses quite diverse functions to regulate different cell types.

Brg function during late T cell development and in mature T cells

We have been interested in studying the roles of BAF complex in the function and gene regulation in different compartments of T cells. The failure of T cell generation in LckCre Brg^{flox/-} mice, where the Brg gene was deleted during the DN stage of thymocyte development [29], prevented us from studying its role in mature T cells. In hope of circumventing this, we crossed CD4Cre transgenic mice with Brg^{flox/-} mice to obtain CD4Cre- Brg^{flox/-} mice; thus Brg is deleted in DP thymocytes and later. Normal thymocyte development was observed when Brg is deleted in the DP stage (our unpublished results). As comforting as it is, this result is surprising in that our previous study demonstrated that Brg is essential for DN thymocyte survival as well as developmental progression [29], indicating a role for Brg in controlling T cell survival and proliferation. This intriguing result provides evidence to support the notion that Brg function is intricately regulated during T cell development; Brg is essential for specific but not all stages of T cell development. Despite the largely normal T cell development in these mice, we observed lymphadenomegaly and splenomegaly with increased lymphocyte counts in older mice. In addition, T cells from these mice gained activated phenotype. One of the possible causes of such a phenotype could be defective Treg function. To test this hypothesis, we analyzed and compared the numbers of Foxp3⁺ Treg cells in the periphery between Brg deficient and WT mice, and no substantial difference was observed. *In vitro*, the suppression activities of BrgKO appeared to be normal. In addition, the expression of Treg “signature” genes was also normal. However, *in vivo*, BrgKO Treg cells did not protect naïve T cell elicited colitis in Rag1^{-/-} mice. This was because Brg deficient Treg cells failed to be maintained in the periphery after transfer. However, conventional T cells lacking the Brg gene were maintained normally in the periphery. These results suggest a specific role for Brg in promoting Treg maintenance *in vivo*. Bone marrow chimera mice, where WT and Brg^{-/-} T cells coexisted in the same physiological environment, were also created. In accordance with our previous results, lower percentage of Treg cells were detected from Brg^{-/-} CD4 T cells compared to those detected from WT cells. Oligo-array assays were performed to identify genes specifically dependent on Brg in Treg cells. Less than 50 genes were significantly affected by Brg deletion. Among them, genes involved in cell survival, metabolism and TGF- β signaling were identified. Therefore, these new findings demonstrated an unexpected, specific role for Brg in controlling Foxp3⁺ Treg peripheral maintenance likely through promoting its survival.

How one CRC specifically regulates gene expression in a cell type and context dependent manner is an important question and warrants further investigation. Revelation of the specific roles of CRC in different cell types will surely enhance the understanding of gene regulation mechanisms and its relevance for normal physiological functions.

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