Symposium 3: Asthma and allergy: From bench to bedside

Deciphering the role of chromatin insulator CTCF in dendritic cells

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CCCTC-binding factor (CTCF) is a highly conserved DNA-binding protein that contains an 11-zinc-finger domain. CTCF shows a genome-wide distribution of DNA occupancy, and 30-60% of its binding is cell type specific. Although CTCF was first described as a transcription factor, and subsequently as a chromatin insulator, recent studies have revealed that CTCF functions to mediate long-range DNA interactions and to identify the borders of topologically associated domains that contribute to three-dimensional chromatin interactions. Topological remodeling of the genome by CTCF can affect the expression of cell differentiation-associated and function-associated genes.

Langerhans cells (LCs) are skin-resident dendritic cells (DCs) that orchestrate skin immunity. Regulation of LC homeostasis depends on several extra- and intra-cellular factors. A possible role of CTCF controlling LC homeostasis and function remains to be determined. Herein, by using conditional gene deletion mouse system, we show that CTCF is critically involved in homeostatic maintenance and migration of LCs in vivo. DC-specific CTCF deletion led to a reduced pool of systemic DCs, and the LC was the most severely affected DC subtype. Decreases in epidermal LC number were specifically associated with defects in self-turnover. Interestingly, CTCF-deficient LCs demonstrated the impaired migration out of the epidermis. Whole transcriptome analyses revealed that genes that promoted cell adhesion were highly expressed, but C-C chemokine receptor 7 was down-regulated in CTCF-depleted LCs. LC-specific CTCF deficient mice showed that hapten-induced CHS responses were more sustained while epicutaneous sensitization to protein antigen was attenuated, indicating that CTCF-dependent LC homeostasis is required for the optimal immune function of LCs in a context-dependent manner. Our results show that CTCF positively regulates the homeostatic pool and the efficient emigration of LCs, which modulates the functional immune network of the skin.

Hematopoiesis involves a series of lineage differentiation programs initiated in hematopoietic stem cells (HSCs) found in bone marrow (BM). To ensure lifelong hematopoiesis, various molecular mechanisms are

needed to maintain the HSC pool. Currently, the role of CTCF in controlling HSC homeostasis is unknown. Using a tamoxifen-inducible CTCF conditional knockout mouse system, we aimed to determine whether CTCF regulates the homeostatic maintenance of HSCs. In adult mice, acute systemic CTCF ablation led to severe BM failure and the rapid shrinkage of multiple c-Kit^{hi} progenitor populations, including Sca-1^{+} HSCs. Similarly, hematopoietic system-confined CTCF depletion caused an acute loss of HSCs and highly increased mortality. Mixed BM chimeras reconstituted with supporting BM demonstrated that CTCF deficiency-mediated HSC depletion has both cell-extrinsic and cell-intrinsic effects. Although c-Kit^{hi} myeloid progenitor cell populations were severely reduced after ablating *Ctcf*, c-Kit^{int} common lymphoid progenitors and their progenies were less affected by the lack of CTCF. Whole-transcriptome microarray and cell cycle analyses indicated that CTCF deficiency results in the enhanced expression of the cell cycle-promoting program, and that CTCF-depleted HSCs express higher levels of reactive oxygen species (ROS). Importantly, *in vivo* treatment with an antioxidant partially rescued c-Kit^{hi} cell populations and their quiescence. Altogether, our results suggest that CTCF is indispensable for maintaining adult HSC pools, likely by regulating ROS-dependent HSC quiescence.

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