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RESEARCH HIGHLIGHT

Long noncoding RNA *lncKdm2b*: A critical player in the maintenance of group 3 innate lymphoid cells

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Innate lymphoid cells (ILCs) represent a heterogeneous population, including both effectors and regulators of innate immunity, inflammation and tissue modeling.[1] ILCs are categorized into three subgroups, ILC1s, ILC2s and ILC3s, based on similarities in phenotypic, ontogenetic and functional characteristics.[2] ILC3s intrinsically require the transcription factor retinoic acid receptor-related orphan receptor γt (ROR γt) for their development and function.[3] In response to specific stimuli, ILC3s produce IL-17 and IL-22 and play a critical role in intestinal mucosal protection, inflammation and innate responses [4] accompanied by a series of pathophysiological changes involving large-scale genetic upregulation and downregulation.[5,6] However, a key challenge remains in understanding the molecular mechanisms underlying the development and maintenance of ILC3s. Recently, progress has been made in identifying a critical factor, long noncoding RNA (lncRNA) *lncKdm2b*, for the regulation of ILC3 function. Liu *et al.*[7] have published their findings in the latest issue of *Nature Immunology*. The identification of *lncKdm2b* along with a comprehensive evaluation of interactions among *lncKdm2b*, the chromatin organizer Satb1, the nuclear remodeling factor complex NURF and Zfp292 has improved our understanding of how this lncRNA is involved in transcriptional programs that modulate ILC3s (Figure 1).[7]

lncRNAs are largely uncharacterized non-protein-coding transcripts longer than 200 nucleotides in length that are implicated in the regulation of a variety of genes and genome activity at multiple levels, including epigenetic mechanisms and nuclear organization, as well as RNA processing, stability and translation.[8] Increasing evidence has indicated that lncRNAs are important factors associated with a wide range of biological processes, including cell proliferation, cell differentiation, apoptosis and self-renewal.[9] *lncKdm2b*, identified by Liu *et al.* is a previously uncharacterized lncRNA that is highly expressed in murine bone marrow, embryo and intestinal lamina propria lymphocytes . Importantly, high expression levels of *lncKdm2b* were observed in ILC3 populations (defined as $Lin^{-}CD45^{+}ROR\gamma t^{+}$), which was further validated using RNA fluorescence *in situ* hybridization (RNA-FISH) and *lncKdm2b* reporter mice. Given that homozygous *lncKdm2b* deficiency is lethal, $lncKdm2b^{flox/flox}$ mice were crossed with Vav-Cre mice to generate a conditional deletion of *lncKdm2b* in hematopoietic cells and their progeny. The data obtained from this model indicate that the expression level of *lncKdm2b* is associated with the proliferation and effector functions of ILC3s without affecting their development and apoptosis.

IL-22 produced by ILC3s is one of the critical effector molecules for mucosal defense against bacterial invasion.[10] As expected, *lncKdm2b* conditional knockout mice, which exhibit reduced numbers of IL-22-producing cells, are very susceptible to *Citrobacter rodentium* infection. In addition to IL-22-mediated protective immune responses, there are other effector molecules, including IL-17 and IL-23, responsible for the maintenance of gut homeostasis and protection against gastrointestinal invasions.[11] Therefore, the production of these effector molecules may be impaired in *lncKdm2b* conditional knockout mice.

lncRNAs play critical roles in spatially orchestrating gene expression through epigenetic, transcriptional and posttranscriptional mechanisms.[12] lncRNAs act as decoy molecules, competing with microRNAs and RNA-binding proteins via target proteins and influence neighboring gene expression in *cis* or *trans*.[13] To address the contribution and mechanism of *lncKdm2b* on the genetic regulatory network in ILC3s, transcriptome microarray and bioinformatics analyses of $lncKdm2b^{+/+}$ ILC3s versus $lncKdm2b^{-/-}$ ILC3s were performed.

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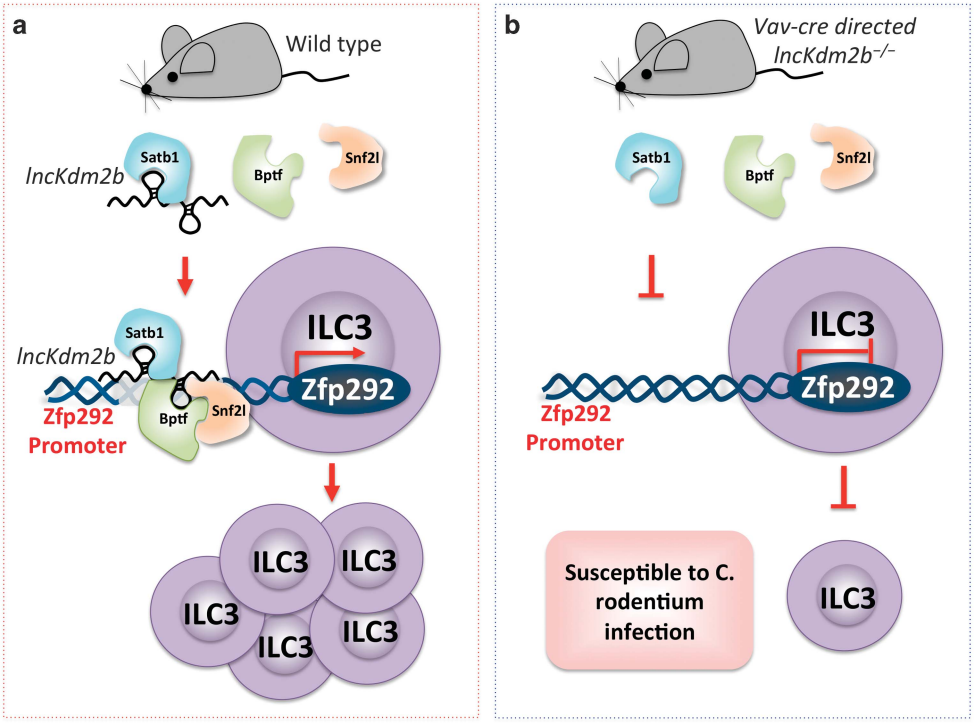

Figure 1 A novel mechanism of *lncKdm2b*-mediated transcriptional regulation implicated in maintaining ILC3 proliferation and function. (**a**) *lncKdm2b* is required for the activation of Zfp292 expression. *lncKdm2b* directly binds to Satb1, which subsequently recruits the NURF complex comprising Bptf and Snf2l. This RNA/protein complex further binds to the Zfp292 promoter to activate Zfp292 expression in ILC3s of wild-type mice. (**b**) Loss of *lncKdm2b* abrogates the recruitment of Satb1 and NURF complex comprising Bptf and Snf2l on the Zfp292 promoter, resulting in impaired number and function of ILC3s. Consequently, Vav-Cre-directed *lncKdm2b* conditional knockout mice are susceptible to *Citrobacter rodentium* infection compared to wild-type mice.

An uncharacterized transcription factor, Zfp292, was identified, which was down-regulated by 5-fold in *lncKdm2b*$^{-/-}$ ILC3s compared to *lncKdm2b*$^{+/+}$ ILC3s. As Zfp292 is an ultimate regulator of *lncKdm2b* in ILC3s, its overexpression offers a feasible and remedial approach to significantly promote ILC3 proliferation and increased resistance to *Citrobacter rodentium* infection. Clearly, there are many regulatory factors upstream of Zfp292. The chromatin organizer Satb1 and the nuclear remodeling factor complex (NURF) are considered to form an indispensable 'bridge' between *lncKdm2b* and Zfp292 in ILC3s. *lncKdm2b* is one of many divergent lncRNAs that are transcribed in the opposite direction relative to their neighboring genes, and thus they influence the expression of neighboring protein-coding genes in *cis*. Interestingly, the neighboring genes of *lncKdm2b* are not influenced in *lncKdm2b*$^{-/-}$ ILC3s. Mechanistically, *lncKdm2b* was found to directly bind to the Satb1 protein and regulate Satb1 expression in *trans* (regulating genes at distal loci) without affecting the neighboring genes in the nuclei of ILC3s. Satb1 is a tissue-specific matrix attachment region-binding protein, which participates in higher order chromatin compaction and tissue-specific gene expression, and it plays a vital role in the regulation of gene expression through chromatin remodeling, histone acetylation and methylation.[14] In ILC3s, Satb1 can regulate Zfp292 expression by recruiting subunits of the NURF chromatin remodeling complex (Bptf and Snf2l) to the Zfp292 promoter. An in-depth study reported that *lncKdm2b*, acting as a scaffold and an amplifier, could recruit and interact with Satb1 and the NURF remodeling complex via distinct binding domains and then bridge these functionally related protein complexes to localize to the Zfp292 promoter to initiate transcription in ILC3s, consistent with the hypothesis of Guttman and Rinn.[15]

These findings reveal a novel *lncKdm2b*-mediated regulatory network involved in the modulation of intestinal homeostasis through the initiation of Zfp292 expression and maintenance of ILC3s, suggesting that *lncKdm2b* may be therapeutically harnessed to regulate ILC3 function during mucosal immune responses and intestinal homeostasis. Taken together, Liu *et al.* have pioneered prospective lncRNA studies by defining how *lncKdm2b* binds or sequesters proteins to activate gene expression in *trans* but not in *cis*. This work highlights two uncharacterized molecules, lncRNAs and Zfp292, in ILC3s and proposes a novel regulatory mechanism underlying ILC3 maintenance. By unraveling the structural and functional relationships and biological relevance, targeting lncRNAs may become a promising approach for treatment.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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