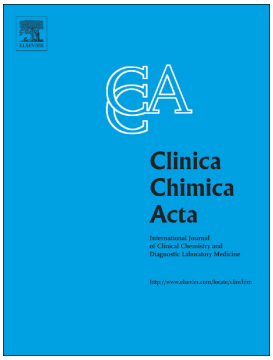
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The correlation between Runx3 and bronchial asthma

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Abstract

Runx3, a member of the Runt-related transcription factor family, has attracted extensive attention due to its important role in the development of immune systems, especially in the differentiation of T cells. Accumulated evidence indicated that altered expression of Runx3 regulates a variety of target genes in different tissues/cells. Studies in animal models suggested that Runx3 may regulate the development of T cell lineage including those of innate lymphoid cells, Treg cells and dendritic cells, which may contribute to the development of hypersensitivity and asthma. Specifically, Runx3 modulates Th1/Th2 balance and hence, the production of interleukins, which induce inflammatory responses. Understanding the roles and mechanisms of Runx3 in the regulation of immune function provides a basis for the design of novel preventive and treatment models for bronchial asthma. This article reviews published data from cell lines, animal models, and patients, concerning the relationship between Runx3 expression alteration and asthma. Epigenetic regulation of Runx3 by DNA hypermethylation and microRNA, and the implication of these pathways in asthma are also discussed.

Keywords: Runx3,asthma

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1. Introduction

As a Runx family member, Runx3 is a crucial transcription factor that regulates a variety of biological processes and orchestrates proper cell fate determination [1,2]. In humans, the gene encoding Runx3 is located in a region of chromosome 1p36, which also contains other genes that have been suggested to induce asthma and hypersensitivity to environmental antigens. Runx3 is found to be expressed in peripheral blood and immune system such as bone marrow, spleen, and thymus, B cells, and T cells [3,4], which may account for its intimate involvement in the development of T-cell differentiation [5].

Pathologically, Runx3 is found to be associated with the occurrence of various human diseases, including gastric and colon cancers and other epithelial diseases such as bronchopulmonary dysplasia [6]. It was reported that the expression levels of Runx3 protein were negatively associated with the HOX transcriptional antisense intergenic RNA (HOTAIR) expression (Pearson coefficient, − 0.501; p = 0.025) in human gastric cancer tissues. Runx3 could inhibit gastric cancer cell migration through inhibition of HOTAIR [7]. Overexpression of HOTAIR can downregulate the expression of specific tumor suppressor genes. HOTAIR can also promote the occurrence and metastasis of various epithelial-derived malignancies, such as breast cancer and colorectal cancer. Through inhibition of HOTAIR expression in tumor, Runx3 can inhibit the growth and invasion ability of tumor cells [8]. Some reports suggests that HDAC1- and SRC-mediated phosphorylation of Runx3 triggered by oxidative stress is associated with the cytoplasmic localization of Runx3 and can lead to Runx3 inactivation, and ultimately, carcinogenesis [9] as well as hypersensitivity [10]. Runx3 gene contains two promoters, P1 and P2, of which P2 is rich in CpG dinucleotides, and Runx3 mRNA is mainly generated by P2 promoter. CpG islands composed of clustered CpG sequences are hypermethylation targets in many genes. Indeed, it was reported that hypermethylation of the CpG-island adjacent to Runx3 P2 promoter in malignant cells of leukemia and cancer could lead to silencing of Runx3 expression [11,12]. The P2 promoter methylation-mediated inhibition of Runx3 gene transcription is considered an important mechanism for Runx3 modulation in cancer cells [13].

Asthma is a chronic inflammatory disease of airways, and a variety of cells representing the innate as well as adaptive immune systems act together with epithelial cells to cause bronchial hyper-reactivity (BHR). Different inflammatory cells, such as epithelial cells and T lymphocytes, release interleukines and other inflammatory mediators to cause muscle contraction and airway constriction. Many nonspecific stimuli such as cold air and exercise are known etiological factors to induce asthma. Acute reactions including mucus overproduction and chronic symptoms including airway wall remodeling/narrowing are frequently found in asthma patients [14]. This review will concentrate on the Runx3 expression alteration and its contribution to the pathogenesis of asthma. The underlying molecular and cellular mechanisms are also reviewed and analyzed.

2. Relationship between Runx3 and asthma

Studies in mouse model have shown that decreased Runx3 expression is associated with spontaneous immunological abnormalities. Zhou et al divided Balb/c mice randomly into the

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control, asthmatic and genistein groups (n=8 for each group). Mice in the asthmatic and genistein groups were sensitized with intraperitoneal injection of 100 μg ovalbumin (OVA). The control group received an intraperitoneal injection of 0.1 ml saline and inhaled aerosolized saline. They found that the expression of nuclear Runx3 protein decreased in mouse lung tissue following induction by OVA. Moreover, after treated by an anti-inflammatory agent genistein, allergic airway inflammation and hypersensitivity were blocked, while the distribution of Runx3 protein in the cell nucleus increased. These results indicate that the abnormal localization of Runx3 protein in lung tissue may contribute to allergic inflammation in mouse asthma model and this molecular mechanism partially explains the inflammatory responses of the airways [10].

Other scholars collected peripheral blood samples from asthma patients and healthy control groups, isolated and cultured CD4+ T cells, and determined the mRNA levels of microRNA-145 and Runx3. Western blotting was used to determine the Runx3 protein level. It was found that Runx3 expression in patients with asthma decreased significantly in CD4+ T cells, while miR-145 increased significantly. Runx3 expression was negatively correlated with microRNA-145 levels. Dual-Luciferase Reporter Assay has shown that Runx3 is a target of microRNA. Upregulation or downregulation of miR-145 level caused Runx3 expression changes in CD4+ T cells and influence the related cytokines. They also found that Runx3 can reverse Th1/Th2 imbalance in asthma patients, suggesting that Runx3 can be used as a biomarker or target for asthma diagnosis or treatment [15]. Singh et al [16] studied how the prenatal environment affected Runx3 expression and the onset of asthma. They showed that when mice were exposed to secondhand smoke during pregnancy, the offspring mice displayed eosinophil infiltration in the lung, resulting in significant BHR. In addition, these changes appeared to be associated with a reduced Runx3 expression in airway epithelium. Overall, these data strongly suggested that reduced expression or abnormal localization of Runx3 plays an important role in the pathogenesis of asthma.

3. Runx3 function and the pathogenesis of bronchial asthma

3.1 The impact of Runx3 on Th1/Th2 balance

CD4+ cells are classified into Th1 and Th2 cells based on their cytokine production in the process of T cell differentiation. Th1 cells mainly produce gamma interferon (IFNγ), while Th2 cells mainly produce interleukin 4 (IL-4) and interleukin 10 (IL-10). These cytokines form a complex network and regulate the body's immune response via a mechanism of mutual antagonism. The function and imbalance of these factors has been linked to the pathogenesis of asthma.

Runx3 may also contribute to the development and function of CD8CTL and Th1 cells [17,18]. Through inhibition of the transcriptional factor ThPOK (T-helper-inducing POZ/Krueppel-like factor), Runx3 participates in MHC I class selection of T cells during the development of CD8+ T cell lineage. Silence of Runx3 expression may inhibit Th1 cytokine secretion, promote the secretion of Th2 cytokines [19]. The expression of Runx3 protein in CD8+ T cells and Th1 cells is high. On the other hand, in the process of CD4+ T and Th2 cell development, the expression of Runx3 protein is limited to Runx3p. This keeps Runx3 protein levels low to maintain T cell plasticity of non-Th1 lineages [20]. In Th1 cells, the upregulation of Runx3d by T-box protein expressed in T cells (T-bet)

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promotes the differentiation of Th1 cells, inducing the secretion of IFNγ [21]. Meanwhile, both the expression and activity of IL-4 was inhibited. Thus, when the expression of Runx3 is inhibited, the imbalance of Th1/Th2 can occur. Qiu et al [22] confirmed that in the CD4+ T cells of asthmatic patients, the expression of Runx3 was decreased, while overexpression of Runx3 could restore the balance of Th1/Th2. Further studies have found that five microRNAs, mir-371, mir-138, mir-544, mir-145 and mir-214, can directly interfere with the function of Runx3's 3’ UTR, leading to reduction of Runx3 expression. Suppressing these 5 microRNAs simultaneously led to increased expression of Runx3 and restoration of the Th1/Th2 balance. However, the specific molecular pathways by which Runx3 affects Th1/Th2 balance has not been fully understood. More studies are required to clarify the underlying mechanisms.

Runx3 is negatively correlated with microRNA not only in asthma, but also in other diseases. D Paudel et al [23] investigated the significance of microRNA (miR)-130b-Runx3 axis in human epithelial ovarian cancer (EOC). MiR-130b expression was downregulated, while RUNX3 mRNA was upregulated, in EOC tissues compared to normal ovarian tissues ($P = 0.001$). Moreover, overexpression of miR-130b reduced the expression of RUNX3 and inhibited cancer cell migration and invasion, whereas knockdown of miR-130b increased the expression of Runx3 and promoted cancer cell migration and invasion. Zhu Y et al found that Runx3 expression was inhibited by miR-210 in response to PQ poisoning. It was concluded that through miR-210-mediated suppression of Runx3, the prolyl hydroxylase domain-containing protein 2 hydroxylation activity was inhibited, and the stability of hypoxia-inducible factor-1α was increased, leading to aggravation of pulmonary fibrosis [24].

3.2 The effect of Runx3 on differentiation of innate lymphoid cells (ILCs)

ILCs are found on the mucosal surface, where they activate immune response, maintain mucosal integrity, and promote the formation of lymphoid tissues. Depending on the specific cytokines, ILCs are divided into three groups: ILC1, ILC2 and ILC3. ILC1 cells can be activated by IL-12, IL-15 and IL-18. These cells express the transcription factor T-bet，a Th1-specific T box transcription factor，and generate Th1 cytokine IFNγ. ILC2 cells can be activated by IL-25 and IL-33, expressing the transcription factor endothelial transcription factor 3 (GATA3), and secret a group of Th2 cytokines IL-5, IL-9 and IL-13. ILC3 cells can be activated by IL-1 depletion and IL-23, and produce IL-17a and/or IL-22 [25].

Runx3 plays an important role in the differentiation of ILCs by affecting the differentiation of CD8+ T cells, Th1 and spleen natural killer (NK) cells [26]. Xiong et al designed an experimental system using a Tomato Red Fluorescent Protein (tRFP)-based reporter for Runx3 expression and a GFP-based Bacterial Artificial Chromosome (BAC) reporter for Thpok expression. They found that in Thpok+/+ mice CD69+ CD4+CD8int MHC-II-restricted cells did not express Runx3 while GATA3 expression was the highest. These results indicated that the expression of Runx3 and GATA3 could be mutually exclusive [27]. Considering that ILCs is related to the expression of both GATA-3 and Runx3, the same transcription network might regulate the differentiation of ILCs. ILC1 cells express a high level of Runx3, which may overwhelm the expression of GATA-3. In contrast, ILC2 cells contain high GATA-3 expression and lose Runx3 expression. In addition, the Runx family members interact with T-bet to control the differentiation of CD4+ T cells. T-bet regulates Runx3 to enhance

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the Th1 differentiation. In the ILC lineage, the possible interaction between T-bet and Runx3 appears to be complicated. Although it remains unclear how T-bet expression is exactly induced, activation of Runx3 expression appeared to be earlier than T-bet expression during ILC differentiation [26].

There is sufficient evidence to show that improper activation of ILCs in lymphoid organs and tissues can also cause inflammatory disease [28]. The potential role of NK cells as a member of the ILC family in asthma and allergic diseases has not been determined. However, there is evidence to show that NK cells participate in the reduction of allergic airway response, especially in the airway mucosal inflammation. Mice lacking CB2 receptors exhibited elevated numbers of pulmonary NK cells yet were resistant to the induction of allergic inflammation as reflected by diminished airway eosinophilia, type 2 cytokine production and mucus secretion after allergen inhalation. These results revealed that NK cells serve to limit ILC2 activation and subsequent allergic airway inflammation [29]. Peripheral blood NK cells in patients with asthma appear to reduce in number, but during the acute onset of asthma, especially in viral infection, there is a significant increase of NK cells, suggesting NK cells play an important role in antiviral host defense. In addition, NK cells are highly activated in severe asthma, and may functionally interact with eosinophils [30]. There is data to indicate that after early exposure to allergens, ILC2 cell activation leads to allergic asthma by activating IL-5 and IL-13 .For example, protein allergens (papain and A. alternata) might activate ILC2 cells indirectly through an effect on lung epithelial cells. The proteolytic injury induces the release of IL-33, which in turn activates ILC2 cells to induce lung eosinophilia and BHR. [31]. In addition, in adult mice and neonatal mice, ILC2 cells upon respiratory infection by viruses such as influenza virus and rhinovirus may be associated with the development of airway hyper responsiveness [32]. This role of ILC2 cells may help to explain how viral exposure causes or exacerbates asthma symptoms in some people. ILC2 cells can also affect the adaptive immune system directly or indirectly by releasing cytokines. ILC2 cells can polarize CD4+ T cells into Th2 cells, which may be an early source of IL-13 [33]. In addition, Oliphant et al found that MHCII-deficient ILC2 cells failed to induce OTIITg T cell proliferation [34]. They also demonstrated that a neutralizing anti-MHCII antibody prevented proliferation of OTIITg T cells co-cultured with peptide-pulsed ILC2s. These data demonstrate that ILC2 may be involved in the expansion and activation of CD4+ T cells.

3.3 The effect of Runx3 on regulatory T cells (Treg cells)

Treg cells negatively regulate the body's immune response of lymphocytes, an action to maintain the tolerance and avoid damage by immune response. This mechanism could be involved in the escape of immune surveillance by tumor cells [35,36]. Research has shown that the function of Treg cells depends on the expression of forkhead box P3 (Foxp3) and the epigenetic regulation of Runx3 is associate with the expression of Foxp3. Khosravi et al applied four different *in vitro* co-culture conditions of mesenchymal stem cells (MSC) with T cells：MSC+mixed lymphocyte response (MLR) condition, MSC+MLR+serum lipase (LPS) condition，and MSC+dendritic cells（DC） condition that are known to allow MSCs to generate iTregs [37]. Depending on the experimental conditions, they observed that variable numbers of iTregs were produced. Looking at the expression of Runx genes (Runx1, Runx3, CBFB), they observed that the transcripts of all these

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genes were changed, leading to expression of the Foxp3. Moreover, in MSC+DC condition they observed the largest alteration in Runx genes expression, suggesting that the more these genes expressed, the more suppressive iTregs are. They also found that the high expression of Runx genes and its combination with the Treg-specific demethylated region (TSDR) resulted in the high and stable expression of Foxp3, further confirming the direct correlation between the induction of Treg and the Runx gene expression. In order to evaluate the importance of these results, they conducted *in vivo* experiments using a more immunogenic experimental model involving transplanting allogeneic skin into immune-competent mice. It was found that Foxp3 mRNA expression increased in MSCs-treated mice. This experiment indicated that MSCs may stabilize Foxp3 via enhancing the expression of Runx. In addition, the imbalance in key transcription factor Runx3, Th-POK and T-bet resulted in the inhibition of Treg/CD4+ T cells [38].

Studies indicated that Treg cells are functionally impaired in people with asthma. Interestingly, this defect of Treg cell function in allergic patients seems to affect only the regulation of Th2 responses. It was reported that in house dust mites allergic children there was a coordinated expression of Foxp3 and GATA transcription factor. Treg cells could inhibit the production of Th1 cytokines but not that of Th2 cytokines. This suggests that Treg cells in allergic patients might promote allergen-specific Th2 responses [31]. In addition, there is a similar phenomenon in which Treg cells were unable to suppress Th2 responses in mice. It was found that in vivo differentiation of Th1 and Th17 cells was suppressed equally well by TIGIT+ vs. TIGIT− Treg cells as judged by IFNγ and IL-17 production. When mice were immunized for induction of Th2-mediated allergic airway inflammation, TIGIT− Treg cells were able to suppress the disease. Consistent with an increase of Th2 cells in the presence of TIGIT+ Treg cells, there were a large number of eosinophils in the bronchio-alveolar lavage of these mice. It was proposed that IL-10 and Fgl2 secreted by TIGIT+ Treg cells may act in concert to suppress both IL-12 and IL-23 production from activated DCs and thereby inhibit the development of both Th1 and Th17 cell responses. TIGIT+ Treg cells therefore shift the balance towards Th2 responses by suppressing pro-inflammatory effector Th1 and Th17 cell responses, but not Th2 cell responses [39]. Laboratory findings support that when the expression of Runx3 is reduced, the inhibition of Treg cells may be weakened, which may trigger the occurrence of allergic diseases such as asthma.

3.4 Runx3 and dendritic cells（DCs）

The abnormal expression of Runx3 can affect the immune function of DCs, impair the balance between immune response and immune tolerance, leading to the occurrence of immune disorders [40]. As antigen-presenting cells, DCs activate and regulate the body's immune response to inhaled allergens in patients with allergic asthma [41]. Dua et al [42] used flow cytometry to determine the DC contents in sputum at 3, 7 and 24 hours after induction with hypertonic saline. The results showed that the myeloid DCs type 2 (mDC2s) in the sputum of asthma patients increased significantly after allergen inhalation, suggesting their involvement in the allergic response to pulmonary allergen. In addition, it was found that CD86 is highly expressed and may promote the differentiation of Th cells into Th2 cells. Jianguo et al studied how CD80 and CD86 downregulation in mDCs affect the Th1/Th2 cytokine expression in mouse asthmatic models. It was found that the mRNA and protein levels of CD80 and CD86 were significantly decreased in mDCs after transfection with relevant siRNA. During co-culture with mDCs, T cell production of IFNγ was significantly

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increased in the siRNA group, while IL-4 production was significantly decreased, suggesting that DCs could regulate Th1/Th2 cytokines production by specific targeting CD80 and CD86 [43].

Another line of evidence in support of Runx3 involvement in DC function comes from Runx3 knockout mouse model. These mice showed an increased serum IgE, an elevated DC surface expression of Fc R by inhaled methacholine, and a hyperresponsiveness to inhaled LPS. Thus, the pathophysiological features found in Runx3 KO mice appeared to recapitulate the clinical symptoms in human asthma. In the absence of Runx3, the bone marrow-derived dendritic cells (BMDC) showed an impaired transcriptional regulation of CCR7 expression, and the lung DC expression of Runx3 was significantly higher than that of the control group. Moreover, Runx3 gene deletion did not only damaged TGF-mediated CCR7 transcription regulation, but resulted in an inhibition of TGF-dependent migration of DC to CCR7 ligand [44]. Takacs et al quantified the mRNA levels of 17 DC-specific transcription factors and observed that the expression levels of 3 transcription factors (Irf4, sp-b and Runx3) in embryonic stem cell-derived dendritic cells（ES-DCs）were lower than that of bone marrow-derived DCs. They used isogene expression screening to explore the role of these transcription factors in the development of mouse ES-DCs, and found that Runx3 improved the maturity of ES-DC. In addition, the ES-DCs activated by runx3 showed an enhanced T cell activation and migration potential [45].

3.5 Runx3 gene hypermethylation in bronchial asthma

It is well recognized that DNA methylation in or near gene promoters regulates gene expression. DNA is methylated by DNA methyltransferase (DNMT), the enzyme catalyzing the transfer of a methyl group from S-adenosylmethionine (SAM) to the 5’ position of a cytosine adjacent to guanine (CpG) [46]. Hypermethylated regions within gene promoters are recognized by methyl-CpG binding domain proteins (MBDs). MBDs recruit histone deacetylases (HDAC) to the promoter region. The histones then become deacetylated and methylated at lysine 9, forming tightly compacted nucleosomes inaccessible to transcription elements (“closed chromatin”) [47]. Yang et al studied a population consisted of inner-city children aged 6 to 12 years with persistent asthma (cases, n = 97) and that without asthma as control (healthy subjects, n = 97)，measured DNA methylation and gene expression in peripheral blood mononuclear cells (PBMCs) using the Illumina’s Infinium Human Methylation 450k BeadChip. They identified methylation alterations in genes related to in T-cell maturation (Runx3), Th2 immunity (IL4), and oxidative stress (catalase), and validated them in an independent asthmatic cohort of children living in the inner city [48]. Clifford et al [49] determined the genome-wide methylation in DNA samples isolated from the airway smooth muscles of patients with asthma using the Illumina 450K Infinium Methylation BeadChip array. Candidate genes were validated by pyrosequencing. RNA was extracted simultaneously for mRNA expression analysis by real-time PCR. Seven hypermethylation sites associated with Runx3, LGALS3BP, ATP11A, ZNF696, KLF6, TBX1, and SPINT2, respectively, were detected and validated. Corresponding reduction in Runx3 expression was found to be associated with the pathogenesis of asthma. It was also observed that following supplementation of diet rich in folic acid for pregnant mice, Runx3 gene became hypermethylated and downregulated in newborn offspring. When treated with demethylation agents, Runx3 gene transcription increased. These results indicate that in the period of embryonic development, dietary factors can increase

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the risk of asthma and other allergic airway diseases through activation of epigenetic mechanisms.

4. Summary

Data from animal model and human population studies implicated Runx3 in the regulation of Th1/Th2 balance and pathogenesis of bronchial asthma. It is likely that Runx3 exerts this effect by modulating the differentiation of multiple immune cell lineages including innate lymphoid cells, Treg cells, as well as dendritic cells. These cells produce different types of cytokines which in turn induce inflammatory responses and airway constriction. Although many aspects of the cellular and molecular mechanisms underlying these biological effects remain to be elucidated, it becomes clear that as a transcriptional factor, Runx3 is capable of regulating a variety of target genes' expression such as CCR7. It is also revealed that Runx3 is subject to epigenetic regulation via DNA hypermethylation- and microRNA-mediated expression inhibition in asthma patients as well as in relevant animal models. Future studies are required to determine how environmental and diet factors associated with increased risk of bronchial asthma may affect the expression and function of Runx3. Better understanding on the roles and pathways of Runx3 in immune system may facilitate the development of novel preventive or therapeutic approaches to improve the management of asthma.

Conflict of interest

The authors confirm that this article content has no conflict of interest.

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Highlights

- Runx3 may play a significant role for the pathogenesis of bronchial asthma.
- Alteration of Runx3 expression may affects the Th1/Th2 balance.
- Runx3 regulates the development of innate lymphoid cells, Treg cells and dendritic cells.
- Runx3 gene is a potential target for asthma prevention and treatment.

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