

Journal Pre-proof

Sirtuins as novel targets in the pathogenesis of airway inflammation in bronchial asthma

Ke Ma, Na Lu, Fei Zou, Fan-Zheng Meng



PII: S0014-2999(19)30622-3

DOI: <https://doi.org/10.1016/j.ejphar.2019.172670>

Reference: EJP 172670

To appear in: *European Journal of Pharmacology*

Received Date: 6 July 2019

Revised Date: 3 September 2019

Accepted Date: 18 September 2019

Please cite this article as: Ma, K., Lu, N., Zou, F., Meng, F.-Z., Sirtuins as novel targets in the pathogenesis of airway inflammation in bronchial asthma, *European Journal of Pharmacology* (2019), doi: <https://doi.org/10.1016/j.ejphar.2019.172670>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2019 Published by Elsevier B.V.

Sirtuins as novel targets in the pathogenesis of airway inflammation in bronchial asthma

Ke Ma¹, Na Lu¹, Fei Zou¹, Fan-Zheng Meng^{1*}

**¹Department of Pediatrics, The First Hospital of Jilin University, Changchun,
Jilin,130021,China**

***Corresponding Author: Fanzheng Meng , Department of Pediatrics, The First Hospital of
Jilin University, Changchun, Jilin, 130021,China Address: No.71, Xinmin Street,
Changchun, Jilin, China Email: fanzheng_m@aliyun.com**

Ke Ma make5528@163.com

Na Lu lu_na245@163.com

Fei Zou feizou5very@sina.com

Abstract

Sirtuins are NAD-dependent class III histone deacetylase, which modulate the epigenetic changes to influence the functions in normal and diseased conditions. Preclinical studies have described an increase in the levels of sirtuin 2 and decrease in the levels of sirtuin 6 in the lungs. Sirtuin 2 exerts proinflammatory actions and hence, its blockers reduce the airway inflammation and symptoms of asthma. On the other hand, sirtuin 6 is anti-inflammatory and its activators produce beneficial actions in asthma. The beneficial effects of sirtuin 6 have been attributed to decrease in acetylation of transcriptional factor GATA3 in the T cells, which is associated with decrease in the T_H2 immune response. However, there seems to be dual role of sirtuin 1 in airway inflammation as its proinflammatory as well as anti-inflammatory actions have been described in asthma. The anti-inflammatory actions of sirtuin 1 have been attributed to decrease in acetylation of GATA3 and inhibition of Akt/NF-kappaB signaling. On the other hand, proinflammatory actions of sirtuin 1 have been attributed to increase in the expression of HIF-1 α and VEGF along with repression of PPAR- γ activity. The present review discusses the role of different sirtuins in the pathogenesis of bronchial asthma. Moreover, it also discusses sirtuin-triggered signaling pathways that may contribute in modulating the disease state of bronchial asthma.

Key Words: sirtuin, bronchial asthma, interleukin, eosinophil, inflammation

1. Introduction

Sirtuins (silent information regulator genes) belong to the family of histone deacetylase (HDAC). Indeed, sirtuins are NAD-dependent class III HDAC and these are further classified into seven types (sirtuins 1 to 7). These different sirtuins have similar catalytic domain and employ NAD⁺ as co-substrate, however, these are different from one another in terms of substrate affinities and sub-cellular localization (Schemies et al., 2010; Vassilopoulos et al., 2011). Being the family members of HDAC, sirtuins primarily modulate the epigenetic changes to induce biological actions (Bosch-Presegué and Vaquero 2015). Epigenetic changes refer to changes in the gene expression without any change in the DNA sequence. A large number of studies have shown that sirtuins regulate the key physiological processes including controlling cellular proliferation, metabolism and aging through transcriptional repression (van de Ven et al., 2017; Gomes et al., 2015). Moreover, their role in the pathophysiology of cardiac diseases (Matsushima and Sadoshima 2015), renal diseases (Morigi et al., 2018), metabolic diseases (Kumar and Lombard 2015), neurodegenerative diseases (Herskovits and Guarente 2013) and cancer (O'Callaghan and Vassilopoulos 2017) has also been increasingly identified.

The role of sirtuins in the development of airway inflammation in bronchial asthma has also been identified (Tang et al., 2017; Colley et al., 2017; Wang et al., 2015). Indeed, it has been shown that sirtuins regulate the different steps of inflammation, particularly T_H2-mediated immune response (Legutko et al., 2011; Hu et al., 2014), whose role in the pathophysiology of bronchial asthma is very well defined (Wegmann et al., 2009). Furthermore, the circadian decline in lung function and exacerbations of respiratory dysfunction has also been associated with sirtuin-mediated post-translation modification of clock gene transcription factors (Yao et al. 2015, Hwang et al., 2014; Sundar et al., 2015). The present review discusses the role of different

sirtuins in the pathogenesis of bronchial asthma. Moreover, it also discusses sirtuin-triggered signaling pathways that may contribute in modulating the disease state of bronchial asthma.

2. Biology of sirtuins

Sirtuins are NAD-dependent class III HDAC as these enzymes have a common requirement of NAD^+ as a cofactor to deacetylate a diverse range of substrates ranging from histone to non-histone proteins including transcriptional factors (Martínez-Redondo and Vaquero 2013). Firstly, sirtuin was discovered in *Saccharomyces cerevisiae* as silent information regulator 2 gene (Sir2) and later, these were discovered in other species including in mammals. All the known sirtuins have an important role in epigenetic regulation i.e. affecting the gene expression without altering the DNA sequence (Bosch-Presegué and Vaquero 2015; Michan and Sinclair 2007). Indeed, deacetylation of histone proteins increases the positive charge on the histone proteins leading to their increased affinity for DNA that result in the formation of a compacted chromatin to repression transcription. Sirtuins-mediated recruitment of other nuclear enzymes to alter histone methylation and DNA CpG methylation also affects gene expression (O'Hagan et al., 2008). Furthermore, sirtuins deacetylate a wide range transcription factors and enzymes including p53, forkhead box subgroup O (FOXOs), peroxisome proliferator-activated receptors (PPARs), NF-kappaB, and DNA-dependent protein kinase to regulate the gene expression (Jęsko and Strosznajder 2016). Interestingly, sirtuins may modulate several cellular processes due to their additional mono-ADP- ribosyltransferase activity (Verheugd et al., 2016).

In the body, the ratio of NAD^+/NADH is sensed by enzymes and accordingly, their enzymatic activity is regulated by this ratio. These enzymes are typically referred to as oxidoreductase enzymes or redox enzymes. Interestingly, sirtuins are non-redox enzymes and their activity is not controlled by NAD^+/NADH ratio or NADH. Rather, NAD^+ is the only

cofactor which seems to influence the enzymatic activity of sirtuins (Anderson et al., 2017). Amongst the seven members, sirtuin 1 is localized in the nucleus and sirtuin 2 is present in the cytoplasm near the microtubules. However, sirtuin 2 has the ability to shuttle between the nucleus and cytoplasm. Sirtuin 3, 4 and 5 are located in the mitochondria and regulate the basic mitochondrial functions including ATP production, metabolism and apoptosis. Sirtuin 6 and 7 are present in the nucleus. Physiologically, sirtuin 6 regulates DNA repair and helps in the maintenance of genomic stability, while sirtuin 7 is present in the nucleoli and works in association with active rRNA genes (Vassilopoulos et al., 2011; Michan and Sinclair 2007).

The interest of scientists in sirtuins was initiated on the basis of the finding that overexpression of these enzymes help in extending the life span possibly by retarding the process of aging (Grabowska et al., 2017). Subsequent studies revealed the wide spectrum of action of these enzymes and scientists delineated the key physiological as well as the pathological functions of sirtuins. The key role of extra-nuclear sirtuins (2, 3, 4, and 5) in regulating metabolic processes and anti-oxidative defense mechanisms has been defined. Indeed, these sirtuins tend to maintain the metabolic homeostasis in response to metabolic stress induced by a large number of factors including overfeeding and obesity (Elkhwanky and Hakkola 2018). Moreover, sirtuins modulate a variety of cellular processes associated with antioxidant and redox signaling (Singh et al., 2018). The key role of sirtuins in cellular proliferation has been delineated and accordingly, studies have shown the key contribution of these enzymes in the pathogenesis of cancers of diverse types (Garcia-Peterson et al., 2017). The role of sirtuins in cancer has been due to the ability of these enzymes to regulate the cellular metabolism, chromatin structure and genomic stability (Chalkiadaki and Guarente 2015). The ability of sirtuins to regulate the transcriptional factors by deacetylation also contributes in controlling the proliferation of cells.

Furthermore, scientists have also explored the role of different sirtuins in the pathogenesis of cardiac diseases (Matsushima and Sadoshima 2015), renal diseases (Morigi et al., 2018), metabolic diseases (Kumar and Lombard 2015), neurodegenerative diseases (Herskovits and Guarente 2013).

Sirtuins have also been projected as key therapeutic targets in the pulmonary diseases due to their ability to modulate the inflammatory processes (Rahman et al., 2012). The deficiency/reduction of sirtuin 1 in the lungs is associated with an increased cell senescence and development of lung diseases including emphysema. Moreover, the activation of sirtuins either by genetic overexpression or using pharmacological activators attenuate stress-induced cellular senescence and protect against emphysema in mice (Gu et al., 2015; Yao et al., 2012, 2014). The key role of sirtuins in the pathogenesis of bronchial asthma is also delineated in a number of studies (Tang et al., 2017; Colley et al., 2017; Wang et al., 2015; Chen et al., 2015).

3. Pharmacological Modulators (activators and inhibitors) of Sirtuins

Scientists have developed various pharmacological modulators of sirtuins to delineate the role of sirtuins in the pathogenesis of diseases and explore their therapeutic potential in ameliorating the disease state (Villalba and Alcáin 2012). Resveratrol is a polyphenol, natural compound which has been employed as activator of sirtuin 1 (Chen et al., 2017; Deus et al., 2017). However, the major limitation of this natural compound is poor bioavailability and scientists have used various formulations of resveratrol to overcome the limitation of bioavailability (Peñalva et al., 2018). In addition, synthetic molecules have also been designed, synthesized and employed as activators of sirtuins 1 which are more potent than resveratrol including STR1720 (Lv et al., 2015), SRT2104 (Jiang et al., 2014; van der Meer et al., 2015) and SRT2379 (Wiewel et al., 2013). These activators have been used in a number of preclinical

studies to delineate the potential role of sirtuin1 in the pathogenesis of different diseases.

SRT2104 has been also used as a drug in a clinical Phase IIa trial in patients and Phase I trial showed this potential drug as safe and well tolerated (Hoffmann et al., 2013; Baksi et al., 2014).

Apart from sirtuin activators, scientists have also developed pharmacological inhibitors of sirtuins to explore their potential in different diseases. Since there is a need of cleavage of nicotinamide from NAD^+ in sirtuin-catalyzed reactions, therefore, scientists have employed nicotinamide as an inhibitor of sirtuin 1 to inhibit the cleavage reactions (Li et al., 2016). The other inhibitors include splitomicin (inhibits sirtuin 1) (Park et al., 2019), sirtinol (inhibits both sirtuin 1 and 2 with IC_{50} values of 131 and 38 μM) (Grozinger et al. 2001; Fong et al., 2014), AGK2 (inhibitor of sirtuin 2) (Yu et al., 2018), cambinol sharing the structural features of sirtinol and splitomicin (inhibits both SIRT1 and SIRT2 with comparable IC_{50}) (Lugrin et al., 2013); suramin (inhibitor of sirtuin 1, 2 and 5) (Vasquez et al., 2017); tenovin 1 (inhibitor of sirtuin 1 and 2) (Marx et al., 2018), tenovin 6 (sirtuin 1 inhibitor) (Yuan et al., 2017); salermide (sirtuin 1 and 2 inhibitor) (Rotili et al., 2012); EX-527 (selective sirtuin 1 inhibitor) (Levine et al., 2016).

4. Molecular Phenotypes of Asthma

In contrast to the earlier views regarding bronchial asthma as a single entity, research has revealed the wide heterogeneity in the asthma patients. This heterogeneity has helped in promoting the concept that asthma consists of multiple phenotypes with their own characteristic features (Wenzel 2012; Ray et al., 2015). Indeed, it has been documented that the current and future therapies for severe asthma management should be based on these molecular phenotypes and biomarkers (Santus et al., 2019). Type 2 molecular phenotype is the well documented form

of asthma in which T_H2 cells-derived cytokines IL-4, -5, and -13 play an important role. Moreover, there is a key involvement of eosinophils, mast cells, IgE, prostaglandin D_2 and type 2 innate lymphoid cells (ILC2). There is a high level of FeNO (fractional excretion of nitric oxide), mainly due to increased iNOS (inducible nitric oxide synthase activity) activity in response to increased levels of type 2 cytokines and eosinophils. Type 2 cytokine-targeted therapies have been clinically successful in the management of type 2 molecular phenotype of asthma. These patients can be managed by specific as well as non-specific anti-inflammatory drugs such as corticosteroids (Kuo et al., 2017). Type 2 phenotype is further subdivided into three subclasses, including early onset or “extrinsic” allergic asthma (atopic), whose characteristic feature is the presence of positive allergy skin tests and increased levels of IgE. There is a comparatively lesser role of IL-5 in comparison to IL-4 and -13 in the development of early onset allergic asthma. In contrast, there is a greater involvement of IL-5 (in comparison to IL-4 and -13) in another subtype, i.e. late (adult)-onset eosinophilic asthma. These patients have low or elevated IgE levels; therefore, the skin allergic test reactivity is commutatively less. However, the eosinophilia is greatest amongst all the subtypes and about 50% of these patients are resistant to steroid therapy. The symptoms are more severe and there is some obstruction in the respiratory system including nasal polyps (Kuruvilla et al., 2019). The third phenotype is the most severe with persistent elevation of eosinophils, neutrophils, IL-4, -5, -13 along with increase in type I cytokine (IFN- γ) and very high levels of FeNO. The contribution of activation of autoimmune system in this type of asthma has been described (Ray et al., 2015).

In contrast in non-type 2 asthma phenotype, type 2 immune pathways do not seem to play a significant role in its pathogenesis. Since there is a very less eosinophils count in the sputum (<2-2.5%) of these patients, this is also termed as non-eosinophilic asthma. Rather, there is a

predominant role of neutrophilic inflammation in this phenotype. Furthermore, there are low levels of FeNO and type 2 cytokines in these patients. Rather, it is proposed that there may be a significant involvement of IL-17 and TNF- α in non-T type 2 phenotype, which may contribute to neutrophilia and steroid resistance (McKinley et al., 2008; Baines et al., 2011). This phenotype is generally associated with risk factors including cigarette smoking, pollution, work-related agents, infections, sinus disease, gastroesophageal reflux and vocal cord dysfunction. The most important feature of this phenotype is that these patients show a poor response to the standard asthma therapy including to inhaled corticosteroids. Therefore, this type of asthma tends to be more severe and difficult to manage (Esteban-Gorgojo et al., 2018).

Another molecular phenotype that has emerged in recent years is obesity-associated asthma. This phenotype has been distinctly identified due to increased tendency of obese persons to develop asthma, particularly women and children. In this type, there is non-significant involvement of type 2 immune response and these patients are characterized by low FeNO, eosinophils, and IgE levels. Moreover, obesity-associated asthma is more severe and difficult to manage (Miethe et al., 2018; Maniscalco et al., 2017; De and Rastogi 2019) (**Table 1**).

5. New Therapeutic Targets in Asthma especially Alteration in Metabolism and Epigenetic Changes

Research studies have identified the emergence of new therapeutic targets that may be pharmacologically modulated for effective management of asthma including peroxisome proliferator-activated receptors, PPAR (Banno et al., 2018); Rho-kinases (Zhang et al., 2019); MAP kinases (Chauhan et al., 2018); Nrf2 as transcriptional factor (Zhang et al., 2019); receptor for advanced glycation end products, RAGE (Brandt and Lewkowich 2019) and transient

receptor potential ion channels (Yao et al., 2019). Apart from these, research studies have also focused on the role of alteration in metabolism in the pathogenesis of asthma. Dysregulation of metabolic pathways affecting metabolism of sphingolipids, arginine, proline, glycerophospholipid and agmatine may also contribute in asthma (Quinn et al., 2017). Moreover, impairment in the cytolytic activity of natural killer cells in asthma persons is attributed to dysregulation of oxidative phosphorylation in these cells (Wu et al., 2018). The importance of dysregulation of metabolism in inflammatory cells is further emphasized by study showing an increase in the expression of enzymes controlling fatty acid oxidation in the bronchial epithelium and inflammatory immune cells infiltrating the respiratory epithelium. Furthermore, pharmacological inhibition of fatty acid oxidation is shown to reduce allergen-induced hyper-responsiveness in airways and decrease the number of inflammatory cells (Al-Khami et al., 2017). Research has shown that environmental pollutants and allergens induce mitochondrial dysfunction by inducing oxidative stress and free radical production. The malfunctioning of mitochondria may alter the bioenergetics of the cell and metabolic profile to favor the induction of systemic inflammation in allergic diseases like asthma (Iyer et al., 2017). The key role of reduced mitochondrial function and low metabolic activity in the development of obesity-associated asthma has also been documented and mitochondrial directed therapies have been found to be effective in managing asthma (Bhatraju and Agrawal 2017). Moreover studies have started revealing that environmental factors such as pollutions, allergens induce epigenetic changes including DNA methylation, histone modifications and small non-coding microRNAs, which are critical in the pathogenesis of asthma (Alizadeh et al., 2017; Vercelli 2016; Potaczek et al., 2017). Indeed, epigenetic changes in the smooth muscle cells of the respiratory airways

may contribute in the pathogenesis of asthma through multiple mechanisms including development of airway inflammation and remodeling (Kaczmarek et al., 2019).

6. Protective role of sirtuins in attenuating airway inflammation in bronchial asthma

6.1 Defective sirtuin 1 increases airway inflammation

Sirtuin 1 is an anti-inflammatory protein and its decreased levels are associated with the development of inflammation (Khader et al., 2017; Chen et al., 2016). Studies have shown that its levels are decreased in the lungs of patients suffering from chronic inflammatory airway disease (Rajendrasozhan et al., 2008). It has been reported that airway allergens-induced development of bronchial asthma is associated with a decrease in the expression of mRNA of sirtuin 1 in the lungs. Moreover, administration of SRT1720, a synthetic activator of sirtuin 1, is shown to attenuate ovalbumin-induced airway inflammation in terms a decrease in the number of eosinophils, bronchoalveolar lavage fluid and the levels of IL-4, IL-5 and IL-13 (Ichikawa et al., 2013). The study performed by Colley et al (2016) described the decrease in protein expression of sirtuin 1 along with a decrease in sirtuin 1 activity in the peripheral blood cells isolated from patients suffering with severe asthma. The decrease in sirtuin 1 activity was directly correlated with the deterioration of parameters assessing the lung functions including a decrease in the forced expiratory volume (FEV1) in asthmatic patients. Moreover, a correlation was also reported between the decrease in sirtuin 1 activity and the increase in the expression of IL-4 in the peripheral blood mononuclear cells along with the increase in the serum levels of IgE. The role of sirtuin 1 in regulating cytokine profile was further supported in *in vitro* studies in which exposure to sirtinol and cambinol increased expression of T_H2-associated cytokines (IL-4 and IL-13) in the blood mononuclear cells of healthy individuals (Colley et al., 2016).

Using 16HBE human lung epithelial cells (in an *in vitro* study), Tang et al (2017) supported the above described findings that a decrease in the sirtuin 1 activity is associated with an increase in the release of proinflammatory cytokines. Treatment of 16HBE human lung epithelial cells with salermide (sirtuin 1 inhibitor) decreased sirtuin activity and increased acetylation p53 (a marker of decreased sirtuin 1 activity) to increase the mRNA and protein expression of IL-6 in a time-dependent manner. RNA interference of sirtuin 1 as well as the knockout of sirtuin 1 also led to an increase in IL-6 expression levels confirming that IL-6 expression is controlled by sirtuin 1 activity (Tang et al., 2017). The precise mechanisms responsible for the decrease in sirtuin 1 activity in asthmatic patients are not explored. Based on the studies describing the destabilization of sirtuin1 in the presence of oxidative stress (Caito et al., 2010) and excessive production of free radicals during bronchial asthma (Sahiner et al., 2011), it may be speculated that allergens (such as cigarette smoke) may trigger the excessive generation of reactive oxygen species, which may reduce the expression levels of sirtuin 1 to activate the T_H2 immune response and initiate the airway inflammatory processes. Similarly, it is hypothesized that a defective c-Jun N-terminal kinase (JNK) 2 signaling in asthmatic persons (Ford et al., 2008) may destabilize sirtuin 1 (Liu et al., 2008) to initiate the T_H2 immune response.

6.2 Overexpression of sirtuin 6 attenuate inflammation

Studies have shown the multiple functions of sirtuin 6 including anti-inflammatory actions through modulation of macrophages (Kugel and Mostoslavsky 2014) and accordingly, its protective role in reducing airway inflammation has been described. The changes in sirtuin 6 expression in human bronchial epithelial cells isolated from asthmatic patients has been documented (Wawrzyniak et al., 2017). In an extensive study, Jang et al (2016) described that

overexpression of sirtuin 6 suppress inflammation in the airways in ovalbumin and house dust mite antigen-challenged mice models. Intratracheal instillation of adenoviruses expressing Sirt6, prior to antigen challenge, significantly reduced the infiltration of inflammatory cells including eosinophils, lymphocytes and macrophages in lung tissues; mucin accumulation in the bronchi; reduced the levels of eotaxin (specific chemoattractant for eosinophils) in bronchoalveolar lavage fluid and lung tissues; the levels of IgE and IgG1 in the serum and airway hyper-responsiveness to cholinergic agent. The reduction in airway hyper-responsiveness was associated with decreases in the transcript levels of CCL2, CCL3, and RANTES in lung tissues along with decreases in the levels of IL-4, IL-5 and IL-13 in the bronchoalveolar lavage fluid and lung tissues. Moreover, there was a significant decrease in the size of the lymph nodes of lungs along with a reduction in the number of IL-4 producing T_H2 cells in sirtuin 6-treated mice suggesting that overexpression of sirtuin 6 may possibly attenuate the T_H2 immune response to attenuate airway inflammation (Jang et al., 2016).

6.3 Possible mechanisms involved in sirtuins-mediated protective actions in airway inflammation

6.3.1 Decreased acetylation of GATA3

GATA3 is a master transcription factor, which is encoded by GATA3 gene and it is found to regulate the T_H2 immune responses in asthma (Zhou and Nie 2015). The GATA3-mediated regulation of T cell response is controlled by acetylation/deacetylation of its gene and hypoacetylation of GATA3 is associated with the reduced functioning of T cells (Yamagata et al., 2000). It has been reported that sirtuin 1 and 6 may modulate the functioning of GATA3 to decrease the T_H2 immune response. Indeed, it has been shown that overexpression of sirtuin 6

normalizes airway allergen-induced increase in the acetylation of GATA3 in the lung tissue. Moreover, it is also revealed that the binding of GATA3 to the promoter region of IL-4 is significantly reduced in sirtuin 6 overexpressing lung tissues. In T-cell lines (*in vitro* studies) also, overexpression of sirtuin 6 is shown to produce deacetylation (hypoacetylation) of GATA3 and decrease the secretion of cytokines from T_H2 cells (Jang et al., 2016). Similarly, the sirtuin 1-mediated regulation of T_H2 immune response is mediated through hypoacetylation of GATA3. In peripheral blood mononuclear cells of healthy individuals, addition of sirtuin 1 activator i.e SRT2172 was shown to increase the expression of T_H2 associated cytokines (IL-4), decrease acetylation of GATA3 along with its decreased binding in the nuclear response elements (Colley et al., 2016). Accordingly, it may be proposed that overexpression of sirtuin 1 and 6 may reduce T_H2 cell differentiation and immune response by inducing deacetylation of GATA3 in T cells (Table 2).

6.3.2 Inhibition of Akt/NF-kappaB signaling

It has been documented that an increase in sirtuin 1 activity may possibly inhibit Akt/NF-kappaB signaling to decrease the expression of IL-6 and inhibit the development of airway inflammation. In an *in vitro* study, the sirtuin 1 inhibitor (salmide) mediated-increase in IL-6 expression in the human lung epithelial cells was associated with the increase in the Akt phosphorylation at Ser473 and Thr308 suggesting the activation of the Akt signaling pathway. However, LY294002 (Akt inhibitor) and BAY11-7082 (I κ B inhibitor) abolished salmide-induced activation of Akt and increase in IL-6 expression in 16HBE cells (Tang et al., 2017). It is known that Akt activates I κ B kinases to increase the phosphorylation of I κ B, which is followed by its degradation in the cytoplasm and translocation of NF- κ B to the nucleus to increase the expression IL-6 (Tak et al., 2001; Bai et al., 2009; Cahill CM and Rogers 2008).

Accordingly, it is proposed that a decrease in the sirtuin 1 activity may activate the Akt/NF-kappaB signaling pathway to increase expression of IL-6, which may participate in the development of airway inflammation in asthmatic patients (**Table 2**).

7. Deleterious effects of sirtuins in airway inflammation in bronchial asthma

7.1 Increase in sirtuin 1 promotes inflammation

In contrast to above described studies, there have been studies describing the inflammation-inducing actions of sirtuin 1 in the ovalbumin-inhaled murine model of airway inflammation (Kim et al., 2010; Legutko et al., 2011; Ahangari et al., 2015). It has been shown that the levels of sirtuin 1 are increased in lungs of ovalbumin-inhaled mice. Administration of sirtinol (non-selective blocker of sirtuin 1) was shown to attenuate the parameters of airway inflammation including mucus secretion, bronchial hyperactivity along with a decrease in the release of T_H2-derived cytokines (IL-4, IL-5 and IL-13) (Kim et al., 2010). Another study has supported the inflammation inducing role of sirtuin 1 in allergic airway disease. Indeed, administration of pharmacological inhibitors of sirtuin 1 including sirtinol and cambinol was shown to inhibit ovalbumin-induced parameters of airway allergy. It was reported that these inhibitors prevent migration and maturation of dendritic cells to suppress the T_H2-mediated immune response and attenuate allergic airway inflammation (Legutko et al., 2011). The study of Ahangari et al (2015) also described an increase in expression of sirtuin 1 in lung tissues in ovalbumin-sensitized mice along with an increase in the secretion of T_H2-derived cytokines. Systemic administration of sirtuin 1 blockers was shown to ameliorate the aeroallergen-induced increase in airway inflammation and release of cytokines in wild mice (Ahangari et al., 2015).

7.2 Sirtuin 2 promotes airway inflammation

Alternative splicing of sirtuin 2 gene leads to formation of three different isoforms of sirtuin 2. The isoforms 1 and 2 are mainly localized in the cytoplasm under the basal, resting state conditions, while isoform 3 in mice (isoform 5 in humans) is mainly localized in the nucleus (Rack et al., 2014). Sirtuin 2 is reported to promote inflammatory processes and macrophages of sirtuin 2-deficient mice are shown to increase the secretion of proinflammatory cytokines (Lo Sasso et al., 2014). A very recent study of Lee et al (2019) has described an increase in the airway inflammatory-related parameters in sirtuin 2 over-expressing transgenic (Sirt2-Tg) mice in response to a combination of three allergens including dust mite, ragweed and *Aspergillus fumigatus*. There was a marked increase in the volume of bronchoalveolar lavage, recruitment of eosinophils, goblet cell hyperplasia and an increase in airway hyperreactivity in Sirt-Tg mice in comparison to normal wild mice. Moreover, there was a marked increase in the levels of CCL17, IL-1, IL-4, IL-5 and TNF- α in the bronchoalveolar lavage in Sirt2-Tg mice. In contrast, airway inflammation-related parameters were significantly less in sirtuin 2 knockout transgenic (Sirt2-KO) mice. Administration of a selective sirtuin 2 antagonist (AGK2) attenuated allergen-induced development of allergic inflammation in wild mice suggesting that the increase in expression of sirtuin 2 may contribute in the pathogenesis of airway inflammation. The authors further delineated through *in vitro* studies that there is upregulation of 3/5 isoform of sirtuin 2 in the lung macrophages that contributes significantly in the development of airway inflammation (Lee et al., 2019).

7.3 Mechanisms involved in inducing deleterious effects of sirtuins in airway inflammation

7.3.1 Sirtuin 1 induces repression of PPAR- γ

PPAR- γ belongs to the family of nuclear receptors and it is involved in the regulation of glucose and lipid metabolism. It has been shown that activation of PPAR- γ induces anti-inflammatory actions (Motoki et al., 2015) and its activation in the dendritic cells leads to inhibition of T_H2 immune response (Nobs et al., 2017). With respect to airway inflammation, it has been described that an increase in the expression of sirtuin 1 represses the PPAR- γ activity in dendritic cells, which is followed by induction of T_H2 immune response leading to release of IL-4, IL-5 and IL-13. Indeed in mice lacking sirtuin 1 in dendritic cells, there was a significant decrease in the development of airway inflammation along with increase in the PPAR- γ activity in dendritic cells (Legutko et al., 2011). There have been other studies showing that sirtuin 1 are capable of repressing PPAR- γ activity (Picard et al., 2004). Therefore, it may be proposed that sirtuin 1-mediated repression of PPAR- γ activity in dendritic cells may lead to overactivation of the T_H2-mediated immune response, which may be critical in the development of bronchial asthma (**Table 2**).

7.3.2 Sirtuin 1 up-regulates expression of hypoxia inducible factor (HIF)-1 α

HIF-1 α is a transcription factor, which responds to low oxygen concentration by increasing the gene expression. However, studies have also shown that this factor also plays a key role of inflammation and its expression is regulated by different cytokines and growth factors (Jung et al., 2003). Moreover, the role of vascular endothelial growth factor (VEGF) in determining the development of asthma has been reported and inhibition of VEGF has been shown to be potential strategy to manage asthma (Lee and Lee 2001). The studies have shown that sirtuin 1 may be involved in the activation of HIF-1 α (Laemmle et al., 2008). In allergic airway model in mice, a parallel increase in the expression of sirtuin 1, HIF-1 α and VEGF in the lung tissues has been documented, which suggests the potential inter-relationship between these

factors. Furthermore, it was shown that administration of sirtinol reduces the levels of HIF-1 α and VEGF in lung tissues and primary tracheal epithelial cells isolated from ovalbumin-inhaled mice. In addition, administration of 2ME2 (HIF-1 α inhibitor) inhibited ovalbumin-induced allergic airway inflammation parameters and the levels of VEGF without significant effect on expression of sirtuin1. It suggests that sirtuin 1 is the upstream mediator and VEGF is the downstream mediator of HIF-1 α . Accordingly, it may be proposed that sirtuin 1 may activate an HIF-1 α transcriptional factor to increase expression of VEGF, which may contribute in the development of bronchial asthma by increasing the secretion of proinflammatory cytokines (Kim et al., 2010) (**Table 2**).

8. Potential Role of Mitochondrial Sirtuins 3,-4 and -5 in Asthma

Sirtuins 3, -4 and -5 are the mitochondrial enzymes that regulate the activity of different metabolic enzymes to affect glucose and fatty acid oxidation (Hirschey et al., 2010; Zhang et al., 2015; Tao et al., 2018). Moreover, it is also well documented that there is a close relationship between metabolism, bioenergetics and immune response (Ganeshan and Chawla 2014). Since sirtuins play an important role in integrating metabolism and immune response; therefore it is possible that the disturbances in metabolic homeostasis may lead to the activation of acute and chronic inflammation (Vachharajani et al., 2017). Although there is no experimental study directly linking the role of mitochondrial sirtuins in the pathogenesis of asthma, yet the close association between metabolism, immune system and asthma (Al-Khami et al., 2017) suggests that these mitochondrial sirtuins may also serve as key targets in asthma. The other reported actions of mitochondrial sirtuins including upregulation of PPAR- γ (Guo et al., 2017); decrease in mitochondrial DNA damage in alveolar epithelial cells (Jablonski et al., 2017; Bindu et al., 2017); decrease in NLRP3 inflammasome activation (Liu et al., 2018); increase in the activation

of superoxide dismutase (Traba et al., 2017) and modulation of innate immune response (Qin et al., 2017) also suggest that the role of mitochondrial sirtuins may be explored in asthma.

There have been evidences documenting the lower levels of sirtuin 4 in obese persons (Barrea et al., 2017) and activation of sirtuin 3 during the prolonged fasting conditions (Traba et al., 2017). Moreover, due to the well documented metabolism improving functions, it is proposed that mitochondrial sirtuins may be potentially exploited to overcome obesity (Newsom et al., 2013). Since the reduction in body weight helps in improving the symptoms of obesity-associated asthma (Özbey et al., 2019), therefore, it may be hypothesized that sirtuins may be particularly relevant in obesity-associated asthma. Nevertheless, experimental studies are needed to verify the key role of mitochondrial sirtuins in asthma.

9. Discussion

Based on the studies performed in the preclinical models of airway inflammation in bronchial asthma, it has been described that there is an increase in the levels of sirtuin 2 (Lee et al., 2019) and a decrease in the levels of sirtuin 6 in lungs (Jang et al., 2016) (**Figure 1**). Sirtuin 2 exerts proinflammatory actions and hence, its blockers are shown to reduce the airway inflammation and symptoms of asthma. On the other hand, sirtuin 6 is anti-inflammatory and its activators are shown to produce beneficial actions in the preclinical model of asthma. The beneficial effects of sirtuin 6 have been attributed to decrease in acetylation of transcriptional factor GATA3 in T cells, which is associated with a decrease in the T_H2 immune response (Jang et al., 2016) (**Figure 1**). However, there seems to be a dual role of sirtuin 1 in airway inflammation as its proinflammatory (Kim et al., 2010; Legutko et al., 2011) as well as anti-inflammatory actions (Ichikawa et al., 2013; Colley et al., 2016) have been described in the

preclinical models of asthma. The anti-inflammatory actions of sirtuin 1 have been attributed to a decrease in decrease in acetylation of GATA3 (Colley et al., 2016) and inhibition of Akt/NF-kappaB signaling (Tang et al., 2017) (**Figure 1**). On the other hand, proinflammatory actions of sirtuin 1 have been attributed to an increase in expression of HIF-1 α and VEGF (Kim et al., 2010) along with repression of PPAR- γ activity (Legutko et al., 2011; Kim et al., 2010). The precise mechanisms responsible for the conflicting/dual actions of sirtuin 1 in airway inflammation are not clear. The dual role of sirtuin 1 i.e. anti-inflammatory and pro-inflammatory actions is not limited to bronchial asthma. Indeed, a number of studies have shown that sirtuin 1 also exhibits dual role in tumorigenesis (Fang and Nicholl 2014). An interesting finding is that both sirtuin 1 activators (Ichikawa et al., 2013) as well as sirtuin 1 blockers (Legutko et al., 2011; Ahangari et al., 2015) are shown to attenuate allergen-induced inflammatory reactions in asthmatic models. More interestingly, both increase in the sirtuin 1 expression (Kim et al., 2010; Ahangari et al., 2015) as well as a decrease in sirtuin 1 expression (Ichikawa et al., 2013) has been documented by different scientists in ovalbumin model, which makes the interpretation of results very difficult. Accordingly, more experimental studies are required to validate the previously reported studies documenting the conflicting results of sirtuin 1 in asthma.

10. Conclusion

The alterations in the levels of sirtuins in lungs may influence the development of airway inflammation. The blockers of sirtuin 2 and activators of sirtuin 6 may be potentially useful in attenuating the symptoms of bronchial asthma. There may be a dual role of sirtuin 1 in airway inflammation as both activators and blockers of sirtuin 1 attenuate allergen-induced inflammatory reactions in asthmatic models. There has not been any research study highlighting

the dual role of sirtuin 1 in airway inflammation. It might be possible that the effects of sirtuin 1 modulators (inhibitors/activators) in airway inflammation are dependent on the stage and severity of the disease. Nevertheless, there is a need of research studies to explore the mechanisms that may contribute in imparting duality to sirtuin 1 in asthma.

References

- Alizadeh Z, Mortaz E, Adcock I, Moin M. Role of Epigenetics in the Pathogenesis of Asthma. *Iran J Allergy Asthma Immunol*. 2017 Apr;16(2):82-91.
- Al-Khami AA, Ghonim MA, Del Valle L, Ibba SV, Zheng L, Pyakurel K, Okpechi SC, Garay J, Wyczzechowska D, Sanchez-Pino MD, Rodriguez PC, Boulares AH, Ochoa AC. Fuelling the mechanisms of asthma: Increased fatty acid oxidation in inflammatory immune cells may represent a novel therapeutic target. *Clin Exp Allergy*. 2017 Sep;47(9):1170-1184.
- Anderson KA, Madsen AS, Olsen CA, Hirschey MD. Metabolic control by sirtuins and other enzymes that sense NAD⁺, NADH, or their ratio. *Biochim Biophys Acta Bioenerg*. 2017 Dec;1858(12):991-998.
- Bai D, Ueno L, Vogt PK. Akt-mediated regulation of NFkappaB and the essentialness of NFkappaB for the oncogenicity of PI3K and Akt. *Int J Cancer*. 2009 Dec 15;125(12):2863-70. doi: 10.1002/ijc.24748.
- Baines KJ, Simpson JL, Wood LG, Scott RJ, Gibson PG. Transcriptional phenotypes of asthma defined by gene expression profiling of induced sputum samples. *J Allergy Clin Immunol* 127: 153–160; e151–e159, 2011

Baksi A, Kraydashenko O, Zalevkaya A, Stets R, Elliott P, Haddad J, Hoffmann E, Vlasuk GP, Jacobson EW. A phase II, randomized, placebo-controlled, double-blind, multi-dose study of SRT2104, a SIRT1 activator, in subjects with type 2 diabetes. *Br J Clin Pharmacol*. 2014 Jul;78(1):69-77.

Banno A, Reddy AT, Lakshmi SP, Reddy RC. PPARs: Key Regulators of Airway Inflammation and Potential Therapeutic Targets in Asthma. *Nucl Receptor Res*. 2018;5. pii: 101306.

Barnes PJ. The cytokine network in asthma and chronic obstructive pulmonary disease. *J Clin Invest* 2008;118:3546-56.

Barrea L, Tarantino G, Somma CD, Muscogiuri G, Macchia PE, Falco A, Colao A, Savastano S. Adherence to the Mediterranean Diet and Circulating Levels of Sirtuin 4 in Obese Patients: A Novel Association. *Oxid Med Cell Longev*. 2017;2017:6101254.

Bhatraju NK, Agrawal A. Mitochondrial Dysfunction Linking Obesity and Asthma. *Ann Am Thorac Soc*. 2017 Nov;14(Supplement_5):S368-S373.

Bindu S, Pillai VB, Kanwal A, Samant S, Mutlu GM, Verdin E, Dulin N, Gupta MP. SIRT3 blocks myofibroblast differentiation and pulmonary fibrosis by preventing mitochondrial DNA damage. *Am J Physiol Lung Cell Mol Physiol*. 2017 Jan 1;312(1):L68-L78.

Bosch-Presegué L, Vaquero A. Sirtuin-dependent epigenetic regulation in the maintenance of genome integrity. *FEBS J*. 2015 May;282(9):1745-67.

Brandt EB, Lewkowich IP. RAGE-induced asthma: a role for the receptor for advanced glycation end products in promoting allergic airway disease. *J Allergy Clin Immunol*. 2019 Jun 25. pii: S0091-6749(19)30823-1.

Cahill CM, Rogers JT. Interleukin (IL) 1 β induction of IL-6 is mediated by a novel phosphatidylinositol 3-kinase-dependent AKT/I κ B kinase α pathway targeting activator protein-1. *J Biol Chem*. 2008 Sep 19;283(38):25900-12. doi: 10.1074/jbc.M707692200. Epub 2008 May 30.

Caito S, Rajendrasozhan S, Cook S, Chung S, Yao H, Friedman A, et al. SIRT1 is a redox-sensitive deacetylase that is post-translationally modified by oxidants and carbonyl stress. *FASEB J* 2010;24:3145-59.

Chalkiadaki A, Guarente L. The multifaceted functions of sirtuins in cancer. *Nat Rev Cancer*. 2015 Oct;15(10):608-24.

Chauhan PS, Singh DK, Dash D, Singh R. Intranasal curcumin regulates chronic asthma in mice by modulating NF- κ B activation and MAPK signaling. *Phytomedicine*. 2018 Dec 1;51:29-38.

Chen AC, Shyu LY, Hsin YL, Chen KM, Lai SC. Resveratrol relieves *Angiostrongylus cantonensis* - Induced meningoencephalitis by activating sirtuin-1. *Acta Trop*. 2017 Sep;173:76-84.

Chen G, Tang J, Ni Z, Chen Q, Li Z, Yang W, Din J, Luo X, Wang X. Antiasthmatic effects of resveratrol in ovalbumin-induced asthma model mice involved in the upregulation of PTEN. *Biol Pharm Bull*. 2015;38(4):507-13. doi: 10.1248/bpb.b14-00610. Epub 2015 Feb 4.

Chen HZ, Wang F, Gao P, Pei JF, Liu Y, Xu TT, Tang X, Fu WY, Lu J, Yan YF, Wang XM, Han L, Zhang ZQ, Zhang R, Zou MH, Liu DP. Age-Associated Sirtuin 1 Reduction in Vascular Smooth Muscle Links Vascular Senescence and Inflammation to Abdominal Aortic Aneurysm. *Circ Res*. 2016 Oct 28;119(10):1076-1088.

Colley T, Mercado N, Kunori Y, Brightling C, Bhavsar PK, Barnes PJ, Ito K. Defective sirtuin-1 increases IL-4 expression through acetylation of GATA-3 in patients with severe asthma. *J Allergy Clin Immunol*. 2016 May;137(5):1595-1597.e7.

De A, Rastogi D. Association of pediatric obesity and asthma, pulmonary physiology, metabolic dysregulation, and atopy; and the role of weight management. *Expert Rev Endocrinol Metab*. 2019 Jun 26:1-15.

Deus CM, Serafim TL, Magalhães-Novais S, Vilaça A, Moreira AC, Sardão VA, Cardoso SM, Oliveira PJ. Sirtuin 1-dependent resveratrol cytotoxicity and pro-differentiation activity on breast cancer cells. *Arch Toxicol*. 2017 Mar;91(3):1261-1278.

Elkhwanky MS, Hakkola J. Extranuclear Sirtuins and Metabolic Stress. *Antioxid Redox Signal*. 2018 Mar 10;28(8):662-676. doi: 10.1089/ars.2017.7270. Epub 2017 Aug 11.

Esteban-Gorgojo I, Antolín-Amérigo D, Domínguez-Ortega J, Quirce S. Non-eosinophilic asthma: current perspectives. *J Asthma Allergy*. 2018 Oct 29;11:267-281.

Fang Y, Nicholl MB. A dual role for sirtuin 1 in tumorigenesis. *Curr Pharm Des*. 2014;20(15):2634-6

Fong Y, Lin YC, Wu CY, Wang HM, Lin LL, Chou HL, Teng YN, Yuan SS, Chiu CC. The antiproliferative and apoptotic effects of sirtinol, a sirtuin inhibitor on human lung cancer cells by modulating Akt/ β -catenin-Foxo3a axis. *Scientific World Journal*. 2014;2014:937051.

Ford J, Ahmed S, Allison S, Jiang M, Milner J. JNK2-dependent regulation of SIRT1 protein stability. *Cell Cycle* 2008;7:3091-7.

- Ganeshan K, Chawla A. Metabolic regulation of immune responses. *Annu Rev Immunol*. 2014;32:609-34. doi: 10.1146/annurev-immunol-032713-120236.
- Garcia-Peterson LM, Wilking-Busch MJ, Ndiaye MA, Philippe CGA, Setaluri V, Ahmad N. Sirtuins in Skin and Skin Cancers. *Skin Pharmacol Physiol*. 2017;30(4):216-224. doi: 10.1159/000477417. Epub 2017 Jul 14.
- Gomes P, Fleming Outeiro T, Cavadas C. Emerging Role of Sirtuin 2 in the Regulation of Mammalian Metabolism. *Trends Pharmacol Sci*. 2015 Nov;36(11):756-768.
- Grabowska W, Sikora E, Bielak-Zmijewska A. Sirtuins, a promising target in slowing down the ageing process. *Biogerontology*. 2017 Aug;18(4):447-476.
- Grozinger CM, Chao ED, Blackwell HE, Moazed D, Schreiber SL. Identification of a class of small molecule inhibitors of the sirtuin family of NAD-dependent deacetylases by phenotypic screening. *J Biol Chem*. 2001 Oct 19;276(42):38837-43. Epub 2001 Aug 1.
- Gu C, Li Y, Xu WL, Yan JP, Xia YJ, Ma YY, Chen C, Wang HJ, Tao HQ. Sirtuin 1 Activator SRT1720 Protects Against Lung Injury via Reduction of Type II Alveolar Epithelial Cells Apoptosis in Emphysema. *COPD*. 2015 Aug;12(4):444-52. doi: 10.3109/15412555.2014.974740.
- Guo X, Yan F, Shan X, Li J, Yang Y, Zhang J, Yan X, Bu P. SIRT3 inhibits Ang II-induced transdifferentiation of cardiac fibroblasts through β -catenin/PPAR- γ signaling. *Life Sci*. 2017 Oct 1;186:111-117.
- Herskovits AZ, Guarente L. Sirtuin deacetylases in neurodegenerative diseases of aging. *Cell Res*. 2013 Jun;23(6):746-58.

Hirschey MD, Shimazu T, Goetzman E, Jing E, Schwer B, Lombard DB, Grueter CA, Harris C, Biddinger S, Ilkayeva OR, Stevens RD, Li Y, Saha AK, Ruderman NB, Bain JR, Newgard CB, Farese RV Jr, Alt FW, Kahn CR, Verdin E. SIRT3 regulates mitochondrial fatty-acid oxidation by reversible enzyme deacetylation. *Nature*. 2010 Mar 4;464(7285):121-5. doi: 10.1038/nature08778.

Hoffmann E, Wald J, Lavu S, Roberts J, Beaumont C, Haddad J, Elliott P, Westphal C, Jacobson E. Pharmacokinetics and tolerability of SRT2104, a first-in-class small molecule activator of SIRT1, after single and repeated oral administration in man. *Br J Clin Pharmacol*. 2013 Jan;75(1):186-96.

Hu T, Fan X, Ma L, Liu J, Chang Y, Yang P, Qiu S, Chen T, Yang L, Liu Z. TIM4-TIM1 interaction modulates Th2 pattern inflammation through enhancing SIRT1 expression. *Int J Mol Med*. 2017 Nov;40(5):1504-1510.

Ichikawa T, Hayashi R, Suzuki K, Imanishi S, Kambara K, Okazawa S, Inomata M, Yamada T, Yamazaki Y, Koshimizu Y, Miwa T, Matsui S, Usui I, Urakaze M, Matsuya Y, Sasahara M, Tobe K. Sirtuin 1 activator SRT1720 suppresses inflammation in an ovalbumin-induced mouse model of asthma. *Respirology*. 2013 Feb;18(2):332-9. doi: 10.1111/j.1440-1843.2012.02284.x.

Iyer D, Mishra N, Agrawal A. Mitochondrial Function in Allergic Disease. *Curr Allergy Asthma Rep*. 2017 May;17(5):29.

Jablonski RP, Kim SJ, Cheres P, Williams DB, Morales-Nebreda L, Cheng Y, Yeldandi A, Bhorade S, Pardo A, Selman M, Ridge K, Gius D, Budinger GRS, Kamp DW. SIRT3 deficiency promotes lung fibrosis by augmenting alveolar epithelial cell mitochondrial DNA damage and apoptosis. *FASEB J*. 2017 Jun;31(6):2520-2532.

Jang HY, Gu S, Lee SM, Park BH. Overexpression of sirtuin 6 suppresses allergic airway inflammation through deacetylation of GATA3. *J Allergy Clin Immunol*. 2016 Nov;138(5):1452-1455.e13.

Jęśko H, Strosznajder RP. Sirtuins and their interactions with transcription factors and poly(ADP-ribose) polymerases. *Folia Neuropathol*. 2016;54(3):212-233.

Jiang M, Zheng J, Peng Q, Hou Z, Zhang J, Mori S, Ellis JL, Vlasuk GP, Fries H, Suri V, Duan W. Sirtuin 1 activator SRT2104 protects Huntington's disease mice. *Ann Clin Transl Neurol*. 2014 Dec;1(12):1047-52.

Jung YJ, Isaacs JS, Lee S, Trepel J, Neckers L. IL-1 β -mediated up-regulation of HIF-1 α via an NF κ B/COX-2 pathway identifies HIF-1 as a critical link between inflammation and oncogenesis. *FASEB J* 2003;17:2115-7.

Kaczmarek KA, Clifford RL, Knox AJ. Epigenetic Changes in Airway Smooth Muscle as a Driver of Airway Inflammation and Remodeling in Asthma. *Chest*. 2019 Apr;155(4):816-824. doi: 10.1016/j.chest.2018.10.038. Epub 2018 Nov 8.

Kaczmarek KA, Clifford RL, Knox AJ. Epigenetic Changes in Airway Smooth Muscle as a Driver of Airway Inflammation and Remodeling in Asthma. *Chest*. 2019 Apr;155(4):816-824.

Khader A, Yang WL, Hansen LW, Rajayer SR, Prince JM, Nicastro JM, Coppa GF, Wang P. SRT1720, a sirtuin 1 activator, attenuates organ injury and inflammation in sepsis. *J Surg Res*. 2017 Nov;219:288-295.

Kim SR, Lee KS, Park SJ, Min KH, Choe YH, Moon H, Yoo WH, Chae HJ, Han MK, Lee YC.

Involvement of sirtuin 1 in airway inflammation and hyperresponsiveness of allergic airway disease. *J Allergy Clin Immunol*. 2010 Feb;125(2):449-460.e14.

Kugel S, Mostoslavsky R. Chromatin and beyond: the multitasking roles for SIRT6. *Trends Biochem Sci* 2014;39:72-81.

Kumar S, Lombard DB. Mitochondrial sirtuins and their relationships with metabolic disease and cancer. *Antioxid Redox Signal*. 2015 Apr 20;22(12):1060-77.

Kuo CS, Pavlidis S, Loza M, Baribaud F, Rowe A, Pandis I, Sousa A, Corfield J, Djukanovic R, Lutter R, Sterk PJ, Auffray C, Guo Y, Adcock IM, Chung KF; U-BIOPRED Study Group. T-helper cell type 2 (Th2) and non-Th2 molecular phenotypes of asthma using sputum transcriptomics in U-BIOPRED. *Eur Respir J*. 2017 Feb 8;49(2). pii: 1602135

Kuruvilla ME, Lee FE, Lee GB. Understanding Asthma Phenotypes, Endotypes, and Mechanisms of Disease. *Clin Rev Allergy Immunol*. 2019 Apr;56(2):219-233.

Laemmle A, Vorburger S, Keogh A, Roh V, Candinas D, Stroka D. Targeting the histone deacetylase-sirt1 for anti-tumor therapy: inhibition of sirt1 down-regulates hif-1. *J Hepatol* 2008;48(suppl):S133-4

Lee YC, Lee HK. Vascular endothelial growth factor in patients with acute asthma. *J Allergy Clin Immunol* 2001;107:1106.

Lee YG, Reader BF, Herman D, Streicher A, Englert JA, Ziegler M, Chung S, Karpurapu M, Park GY, Christman JW, Ballinger MN. Sirtuin 2 enhances allergic asthmatic inflammation. *JCI Insight*. 2019 Feb 21;4(4).

Legutko A, Marichal T, Fiévez L, Bedoret D, Mayer A, de Vries H, Klotz L, Drion PV, Heirman C, Cataldo D, Louis R, Thielemans K, Andris F, Leo O, Lekeux P, Desmet CJ, Bureau F. Sirtuin 1 promotes Th2 responses and airway allergy by repressing peroxisome proliferator-activated receptor- γ activity in dendritic cells. *J Immunol*. 2011 Nov 1;187(9):4517-29. doi: 10.4049/jimmunol.1101493. Epub 2011 Sep 23.

Levine MH, Wang Z, Xiao H, Jiao J, Wang L, Bhatti TR, Hancock WW, Beier UH. Targeting Sirtuin-1 prolongs murine renal allograft survival and function. *Kidney Int*. 2016 May;89(5):1016-1026.

Li WY, Ren JH, Tao NN, Ran LK, Chen X, Zhou HZ, Liu B, Li XS, Huang AL, Chen J. The SIRT1 inhibitor, nicotinamide, inhibits hepatitis B virus replication in vitro and in vivo. *Arch Virol*. 2016 Mar;161(3):621-30.

Liu P, Huang G, Wei T, Gao J, Huang C, Sun M, Zhu L, Shen W. Sirtuin 3-induced macrophage autophagy in regulating NLRP3 inflammasome activation. *Biochim Biophys Acta Mol Basis Dis*. 2018 Mar;1864(3):764-777.

Liu W, Liang Q, Balzar S, Wenzel S, Gorska M, Alam R. Cell-specific activation profile of extracellular signal-regulated kinase 1/2, Jun N-terminal kinase, and p38 mitogen-activated protein kinases in asthmatic airways. *J Allergy Clin Immunol* 2008;121:893-902.e2.

Lo Sasso G, et al. SIRT2 deficiency modulates macrophage polarization and susceptibility to experimental colitis. *PLoS ONE*. 2014;9(7):e103573.

Lugrin J, Ciarlo E, Santos A, Grandmaison G, dos Santos I, Le Roy D, Roger T. The sirtuin inhibitor cambinol impairs MAPK signaling, inhibits inflammatory and innate immune responses and protects from septic shock. *Biochim Biophys Acta*. 2013 Jun;1833(6):1498-510.

Lv C, Hu HY, Zhao L, Zheng H, Luo XZ, Zhang J. Intrathecal SRT1720, a SIRT1 agonist, exerts anti-hyperalgesic and anti-inflammatory effects on chronic constriction injury-induced neuropathic pain in rats. *Int J Clin Exp Med*. 2015 May 15;8(5):7152-9. eCollection 2015.

Maniscalco M, Paris D, Melck DJ, D'Amato M, Zedda A, Sofia M, Stellato C, Motta A. Coexistence of obesity and asthma determines a distinct respiratory metabolic phenotype. *J Allergy Clin Immunol*. 2017 May;139(5):1536-1547.e5. doi: 10.1016/j.jaci.2016.08.038. Epub 2016 Oct 14.

Martínez-Redondo P, Vaquero A. The diversity of histone versus nonhistone sirtuin substrates. *Genes Cancer*. 2013 Mar;4(3-4):148-63.

Marx C, Marx-Blümel L, Lindig N, Thierbach R, Hoelzer D, Becker S, Wittig S, Lehmann R, Slevogt H, Heinzel T, Wang ZQ, Beck JF, Sonnemann J. The sirtuin 1/2 inhibitor tenovin-1 induces a nonlinear apoptosis-inducing factor-dependent cell death in a p53 null Ewing's sarcoma cell line. *Invest New Drugs*. 2018 Jun;36(3):396-406.

Matsushima S, Sadoshima J. The role of sirtuins in cardiac disease. *Am J Physiol Heart Circ Physiol*. 2015 Nov;309(9):H1375-89.

McKinley L, Alcorn JF, Peterson A, Dupont RB, Kapadia S, Logar A, Henry A, Irvin CG, Piganelli JD, Ray A, Kolls JK. TH17 cells mediate steroid-resistant airway inflammation and airway hyperresponsiveness in mice. *J Immunol* 181: 4089–4097, 2008.

Michan S, Sinclair D. Sirtuins in mammals: insights into their biological function. *Biochem J*. 2007 May 15;404(1):1-13.

Miethe S, Guarino M, Alhamdan F, Simon HU, Renz H, Dufour JF, Potaczek DP, Garn H. Effects of obesity on asthma: immunometabolic links. *Pol Arch Intern Med*. 2018 Aug 31;128(7-8):469-477. doi: 10.20452/pamw.4304. Epub 2018 Jul 27.

Morigi M, Perico L, Benigni A. Sirtuins in Renal Health and Disease. *J Am Soc Nephrol*. 2018 Jul;29(7):1799-1809.

Motoki T, Kurobe H, Hirata Y, Nakayama T, Kinoshita H, Rocco KA, Sogabe H, Hori T, Sata M, Kitagawa T. PPAR- γ agonist attenuates inflammation in aortic aneurysm patients. *Gen Thorac Cardiovasc Surg*. 2015 Oct;63(10):565-71.

Newsom SA, Boyle KE, Friedman JE. Sirtuin 3: A major control point for obesity-related metabolic diseases? *Drug Discov Today Dis Mech*. 2013 Jun 1;10(1-2):e35-e40.

Nobs SP, Natali S, Pohlmeier L, Okreglicka K, Schneider C, Kurrer M, Sallusto F, Kopf M. PPAR γ in dendritic cells and T cells drives pathogenic type-2 effector responses in lung inflammation. *J Exp Med*. 2017 Oct 2;214(10):3015-3035

O'Callaghan C, Vassilopoulos A. Sirtuins at the crossroads of stemness, aging, and cancer. *Aging Cell*. 2017 Dec;16(6):1208-1218.

O'Hagan HM, Mohammad HP, Baylin SB. Double strand breaks can initiate gene silencing and SIRT1-dependent onset of DNA methylation in an exogenous promoter CpG island. *PLoS Genet*. 2008 Aug 15;4(8):e1000155.

Özbey Ü, Balaban S, Sözüner ZÇ, Uçar A, Mungan D, Mısırlıgil Z. The effects of diet-induced weight loss on asthma control and quality of life in obese adults with asthma: a randomized controlled trial. *J Asthma*. 2019 Mar 23;1-9

Park JA, Park S, Park WY, Han MK, Lee Y. Splitomicin, a SIRT1 Inhibitor, Enhances Hematopoietic Differentiation of Mouse Embryonic Stem Cells. *Int J Stem Cells*. 2019 Mar 30;12(1):21-30.

Peñalva R, Morales J, González-Navarro CJ, Larrañeta E, Quincoces G, Peñuelas I, Irache JM. Increased Oral Bioavailability of Resveratrol by Its Encapsulation in Casein Nanoparticles. *Int J Mol Sci*. 2018 Sep 18;19(9). pii: E2816. doi: 10.3390/ijms19092816.

Picard, F., M. Kurtev, N. Chung, A. Topark-Ngarm, T. Senawong, R. Machado De Oliveira, M. Leid, M. W. McBurney, and L. Guarente. 2004. Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR- γ . *Nature* 429: 771– 776.

Potaczek DP, Harb H, Michel S, Alhamwe BA, Renz H, Tost J. Epigenetics and allergy: from basic mechanisms to clinical applications. *Epigenomics*. 2017 Apr;9(4):539-571.

Qin K, Han C, Zhang H, Li T, Li N, Cao X. NAD⁺ dependent deacetylase Sirtuin 5 rescues the innate inflammatory response of endotoxin tolerant macrophages by promoting acetylation of p65. *J Autoimmun*. 2017 Jul;81:120-129.

Quinn KD, Schedel M, Nkrumah-Elie Y, Joetham A, Armstrong M, Cruickshank-Quinn C, Reisdorph R, Gelfand EW, Reisdorph N. Dysregulation of metabolic pathways in a mouse model of allergic asthma. *Allergy*. 2017 Sep;72(9):1327-1337.

Rack JG, VanLinden MR, Lutter T, Aasland R, Ziegler M. Constitutive nuclear localization of an alternatively spliced sirtuin-2 isoform. *J Mol Biol.* 2014;426(8):1677–1691.

Rahman I, Kinnula VL, Gorbunova V, Yao H. SIRT1 as a therapeutic target in inflammaging of the pulmonary disease. *Prev Med.* 2012 May;54 Suppl:S20-8. doi: 10.1016/j.ypmed.2011.11.014. Epub 2011 Dec 8.

Rajendrasozhan S, Yang SR, Kinnula VL, Rahman I. SIRT1, an antiinflammatory and antiaging protein, is decreased in lungs of patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med.* 2008 Apr 15;177(8):861-70.

Ray A, Oriss TB, Wenzel SE. Emerging molecular phenotypes of asthma. *Am J Physiol Lung Cell Mol Physiol.* 2015 Jan 15;308(2):L130-40. doi: 10.1152/ajplung.00070.2014. Epub 2014 Oct 17.

Rotili D, Tarantino D, Nebbioso A, Paolini C, Huidobro C, Lara E, Mellini P, Lenoci A, Pezzi R, Botta G, Lahtela-Kakkonen M, Poso A, Steinkühler C, Gallinari P, De Maria R, Fraga M, Esteller M, Altucci L, Mai A. Discovery of salermide-related sirtuin inhibitors: binding mode studies and antiproliferative effects in cancer cells including cancer stem cells. *J Med Chem.* 2012 Dec 27;55(24):10937-47.

Sahiner UM, Birben E, Erzurum S, Sackesen C, Kalayci O. Oxidative stress in asthma. *World Allergy Organ J* 2011;4:151-8.

Santus P, Saad M, Damiani G, Patella V, Radovanovic D. Current and future targeted therapies for severe asthma: Managing treatment with biologics based on phenotypes and biomarkers. *Pharmacol Res.* 2019 Jun 4;146:104296.

Schemies J, Uciechowska U, Sippl W, Jung M. NAD(+) -dependent histone deacetylases (sirtuins) as novel therapeutic targets. *Med Res Rev.* 2010 Nov;30(6):861-89. doi: 10.1002/med.20178.

Singh CK, Chhabra G, Ndiaye MA, Garcia-Peterson LM, Mack NJ, Ahmad N. The Role of Sirtuins in Antioxidant and Redox Signaling. *Antioxid Redox Signal.* 2018 Mar 10;28(8):643-661.

Sundar IK, Yao H, Sellix MT, Rahman I. Circadian molecular clock in lung pathophysiology. *Am J Physiol Lung Cell Mol Physiol.* 2015 Nov 15;309(10):L1056-75.

Tak PP, Firestein GS: NF-kappaB: a key role in inflammatory diseases. *J Clin Invest* 2001;107:7-11.

Tang L, Chen Q, Meng Z, Sun L, Zhu L, Liu J, Hu J, Ni Z, Wang X. Suppression of Sirtuin-1 Increases IL-6 Expression by Activation of the Akt Pathway During Allergic Asthma. *Cell Physiol Biochem.* 2017;43(5):1950-1960. doi: 10.1159/000484119. Epub 2017 Oct 20.

Tao J, Zhang J, Ling Y, McCall CE, Liu TF. Mitochondrial Sirtuin 4 Resolves Immune Tolerance in Monocytes by Rebalancing Glycolysis and Glucose Oxidation Homeostasis. *Front Immunol.* 2018 Mar 9;9:419.

Traba J, Geiger SS, Kwarteng-Siaw M, Han K, Ra OH, Siegel RM, Gius D, Sack MN. Prolonged fasting suppresses mitochondrial NLRP3 inflammasome assembly and activation via SIRT3-mediated activation of superoxide dismutase 2. *J Biol Chem.* 2017 Jul 21;292(29):12153-12164.

Vachharajani VT, Liu T, Wang X, Hoth JJ, Yoza BK, McCall CE. Sirtuins Link Inflammation and Metabolism. *J Immunol Res.* 2016;2016:8167273.

van de Ven RAH, Santos D, Haigis MC. Mitochondrial Sirtuins and Molecular Mechanisms of Aging. *Trends Mol Med*. 2017 Apr;23(4):320-331.

van der Meer AJ, Scicluna BP, Moerland PD, Lin J, Jacobson EW, Vlasuk GP, van der Poll T. The Selective Sirtuin 1 Activator SRT2104 Reduces Endotoxin-Induced Cytokine Release and Coagulation Activation in Humans. *Crit Care Med*. 2015 Jun;43(6):e199-202.

Vasquez MC, Beam M, Blackwell S, Zuzow MJ, Tomanek L. Sirtuins regulate proteomic responses near thermal tolerance limits in the blue mussels *Mytilus galloprovincialis* and *Mytilus trossulus*. *J Exp Biol*. 2017 Dec 1;220(Pt 23):4515-4534.

Vassilopoulos A, Fritz KS, Petersen DR, Gius D. The human sirtuin family: evolutionary divergences and functions. *Hum Genomics*. 2011 Jul;5(5):485-96.

Vassilopoulos A, Fritz KS, Petersen DR, Gius D. The human sirtuin family: evolutionary divergences and functions. *Hum Genomics*. 2011 Jul;5(5):485-96.

Vercelli D. Does epigenetics play a role in human asthma? *Allergol Int*. 2016 Apr;65(2):123-126.

Verheugd P, Bütepage M, Ecker L, Lüscher B. Players in ADP-ribosylation: Readers and Erasers. *Curr Protein Pept Sci*. 2016;17(7):654-667.

Villalba JM, Alcaín FJ. Sirtuin activators and inhibitors. *Biofactors*. 2012 Sep-Oct;38(5):349-59.

Wawrzyniak P, Wawrzyniak M, Wanke K, Sokolowska M, Bendelja K, Rückert B, Globinska A, Jakiela B, Kast JJ, Idzko M, Akdis M, Sanak M, Akdis CA. Regulation of bronchial epithelial barrier integrity by type 2 cytokines and histone deacetylases in asthmatic patients. *J Allergy Clin Immunol*. 2017 Jan;139(1):93-103. doi: 10.1016/j.jaci.2016.03.050. Epub 2016 May 11.

Wegmann M. Th2 cells as targets for therapeutic intervention in allergic bronchial asthma.

Expert Rev Mol Diagn. 2009 Jan;9(1):85-100.

Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. Nat Med.

2012 May 4;18(5):716-25. doi: 10.1038/nm.2678.

Wiewel MA, van der Meer AJ, Haddad J, Jacobson EW, Vlasuk GP, van der Poll T. SRT2379, a small-molecule SIRT1 activator, fails to reduce cytokine release in a human endotoxemia model.

Crit Care. 2013; 17(Suppl 4): P8.

Wu H, Bi J, Wu G, Zheng C, Lu Z, Cui L, Wan X. Impaired cytolytic activity of asthma-associated natural killer cells is linked to dysregulated transcriptional program in energy metabolism. Mol Immunol. 2018 Sep;101:514-520.

Yamagata T, Mitani K, Oda H, Suzuki T, Honda H, Asai T, et al. Acetylation of GATA-3 affects T-cell survival and homing to secondary lymphoid organs. EMBO J 2000;19:4676-87.

Yao H, Chung S, Hwang JW, Rajendrasozhan S, Sundar IK, Dean DA, McBurney MW, Guarente L, Gu W, Rönty M, Kinnula VL, Rahman I. SIRT1 protects against emphysema via FOXO3-mediated reduction of premature senescence in mice. J Clin Invest. 2012 Jun;122(6):2032-45. doi: 10.1172/JCI60132. Epub 2012 May 1.

Yao H, Sundar IK, Ahmad T, Lerner C, Gerloff J, Friedman AE, Phipps RP, Sime PJ, McBurney MW, Guarente L, Rahman I. SIRT1 protects against cigarette smoke-induced lung oxidative stress via a FOXO3-dependent mechanism. Am J Physiol Lung Cell Mol Physiol. 2014 May 1;306(9):L816-28. doi: 10.1152/ajplung.00323.2013. Epub 2014 Mar 14.

Yao H, Sundar IK, Huang Y, Gerloff J, Sellix MT, Sime PJ, Rahman I. Disruption of sirt1-mediated regulation of circadian molecular clock and inflammation in copd. *Am J Respir Cell Mol Biol* 2015 Dec;53(6):782-92

Yao L, Chen S, Tang H, Huang P, Wei S, Liang Z, Chen X, Yang H, Tao A, Chen R, Zhang Q. Transient Receptor Potential Ion Channels Mediate Adherens Junctions Dysfunction in a Toluene Diisocyanate-Induced Murine Asthma Model. *Toxicol Sci.* 2019 Mar 1;168(1):160-170.

Yu HB, Jiang H, Cheng ST, Hu ZW, Ren JH, Chen J. AGK2, A SIRT2 Inhibitor, Inhibits Hepatitis B Virus Replication In Vitro And In Vivo. *Int J Med Sci.* 2018 Sep 7;15(12):1356-1364.

Yuan H, Tan B, Gao SJ. Tenovin-6 impairs autophagy by inhibiting autophagic flux. *Cell Death Dis.* 2017 Feb 9;8(2):e2608.

Zhang JH, Yang X, Chen YP, Zhang JF, Li CQ. Nrf2 Activator RTA-408 Protects Against Ozone-Induced Acute Asthma Exacerbation by Suppressing ROS and $\gamma\delta$ T17 Cells. *Inflammation.* 2019 Jun 29. doi: 10.1007/s10753-019-01046-6. [Epub ahead of print]

Zhang W, Li X, Zhang Y. Rho-kinase inhibitor attenuates airway mucus hypersecretion and inflammation partly by downregulation of IL-13 and the JNK1/2-AP1 signaling pathway. *Biochem Biophys Res Commun.* 2019 Jun 21. pii: S0006-291X(19)31208-2.

Zhang Y, Bharathi SS, Rardin MJ, Uppala R, Verdin E, Gibson BW, Goetzman ES. SIRT3 and SIRT5 regulate the enzyme activity and cardiolipin binding of very long-chain acyl-CoA dehydrogenase. *PLoS One.* 2015 Mar 26;10(3):e0122297.

Zhou W, Nie X. Afzelin attenuates asthma phenotypes by downregulation of GATA3 in a murine model of asthma. *Mol Med Rep*. 2015 Jul;12(1):71-6.

Zhu J, Min B, Hu-Li J, Watson CJ, Grinberg A, Wang Q, et al. Conditional deletion of Gata3 shows its essential function in TH1-TH2 responses. *Nat Immunol* 2004;5: 1157-65.

Figure Legend

Figure 1: Exposure to allergens may lead to the changes in expression/enzymatic activities of sirtuins, possibly through peroxynitrite-mediated increase in NF-kappaB activation (though not verified experimentally). The changes in the expression/activity of sirtuins may lead to the activation of T_H2 immune response through hyperacetylation of GATA3, which is followed by increase in the release of type 2 cytokines IL-4, -5 and -13. The precise involvement of sirtuins downstream of T_H2 cells in airway inflammation is not explored. The cytokines may induce multiple changes to trigger inflammatory reactions in the respiratory airways including the activation of B cells to increase IgE production, hyper proliferation of smooth muscle cells to induce bronchial hyperactivity and recruitment of eosinophils. Moreover, sirtuins-mediated changes in the transcriptional activity may induce Akt activation followed by Ikb degradation to induce activation of NF-kappaB. However, the role of sirtuins in non type 2 asthma phenotypes (non-allergic and obesity-associated) is not experimentally verified.

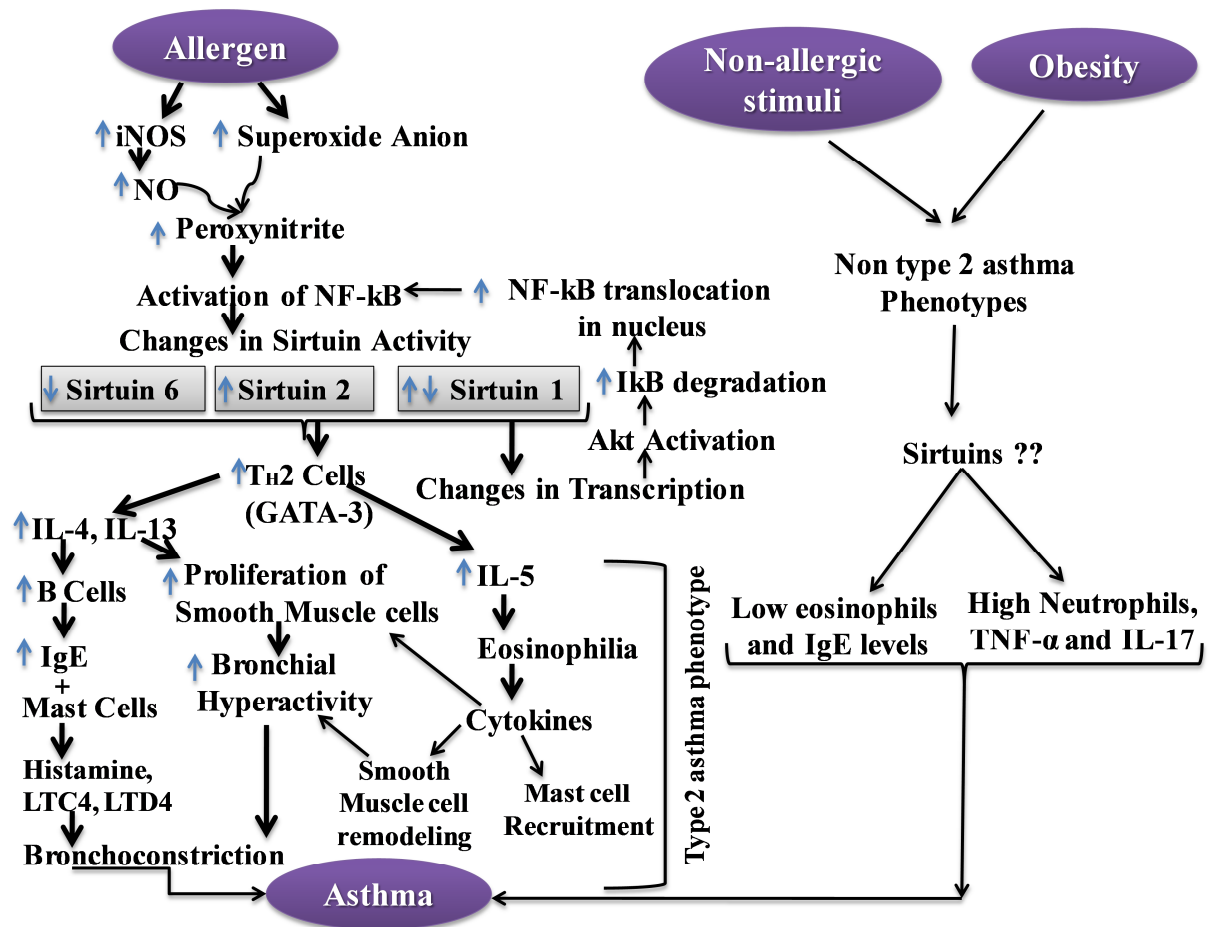


Figure 1

Table 1: Summarized description of different molecular phenotypes of asthma

S. No	Molecular Phenotypes	Characteristic Features
1.	Type 2 Asthma	
	• Key role of T_H2 immune system • Role of eosinophils, mast cells, IgE, ILC2 cells • High levels of FeNO • Respond to conventional anti-inflammatory therapy	
1a	Early onset allergic asthma	• Presence of positive allergy skin tests and increased IgE • More role of IL-4 and -13
1b	Late (adult)-onset eosinophilic asthma	• Less reactivity to skin allergy test and normal to mild increase in IgE • More role of IL-5 • Eosinophilia is more marked • Relative resistance to steroid therapy
1c	Most severe with component of autoimmunity	• Elevation of eosinophils, neutrophils, IL-4,-5,-13 • Increase in type I cytokine (IFN-γ) • Very high levels of FeNO
2.	Non-type 2 asthma (Non-eosinophilic asthma)	
	• No role of T_H2 immune system • Less eosinophils, low IgE; more neutrophils • Less type 2 cytokines IL-4,-5,-13; low FeNO • More IL-17 and TNF-α • Resistant to steroid therapy	
3.	Obesity-associated asthma	
	• No role of T_H2 immune system • Less eosinophils, low IgE • Less type 2 cytokines IL-4,-5,-13; low FeNO	

Table 2: Summarization of different targets modulated in response to change in the expression of sirtuins to induce protective and deleterious effects in airway inflammation

S. No	Sirtuins	Targets	Effects	References
Sirtuins-mediated protective actions in airway inflammation				
1.	Sirtuin 1 and 6	GATA3	Overexpression of sirtuin 1 and 6 produces deacetylation of GATA3 to decrease the secretion of cytokines from T _H 2 cells	Jang et al., 2016; Colley et al., 2016
2.	Sirtuin 1	Akt	Decrease in sirtuin 1 promotes Akt phosphorylation followed by activation of NF-kappaB signaling to promote inflammation	Tang et al., 2017
Deleterious effects of sirtuins in airway inflammation				
3.	Sirtuin 1	PPAR- γ	Increase in sirtuin 1 represses PPAR- γ activity to induce T _H 2 immune response and increases secretion of cytokines	Legutko et al., 2011
4.	Sirtuin 1	HIF-1 α	Increase in sirtuin 1 activates HIF-1 α to increase the expression of VEGF, which promotes airway inflammation	Kim et al., 2010

Author Agreement

It is verified that all the authors have read and approved the final version of the manuscript. It is declared that this paper is authors work and is not submitted elsewhere for publication.

Journal Pre-proof