Asthma

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The concept of asthma disease has been around since 400 BC (Asthma & Allergy 2018) and yet, there is no cure for it. It was not until the 20th century when asthma was specifically classified as an inflammatory disorder, but its severity was underestimated. Currently, the chronic inflammation and malfunctioning airway classify the disease as a major noncommunicable disease that affects both children and adults (WHO, 2022). Worldwide, in 2019 asthma affected 262 million people; 455,000 had died. Unfortunately, the condition continues to burden many children and adults (WHO, 2022).

Specifically in the United States, data collected by the NHIS in 2022 revealed that 26 million people suffer from asthma (Goff, 2023). Of this, 21 million were adults. There needs to be progress made in this field of study as scientists continue to battle the ongoing challenge of defining asthma. That is why asthma research supported by a well-financed foundation should be prioritized. In doing so, innovative methods can be produced to identify, classify, and diagnose asthma at an earlier stage. Early, accurate diagnosis nurtures optimal disease management, precision medicine and reduced asthma-attributable health burdens.

Asthma is a heterogeneous disease and so its severity and risk factors range between people. However, each day, 10 people in the U.S. die from asthma (Goff, 2023). Most deaths are likely Black and/or females. Moreover, those who are at higher risk for developing asthma include Black and female populations. In fact, around 9.7% adult-females have asthma (Goff, 2023). And Black children are two times as likely to suffer from asthma than white children. In the U.S, Black people are also three times more likely to die from asthma. And despite the advancements in the diagnosis and care of asthma, a large population continues to suffer. To reduce these rates, it is important to further asthma research. While patterns of diagnosis including age, gender and race are important to characterize asthma, asthma cannot be understood without study of its cause. Like asthma's heterogeneity in who gets the diseases, asthma is caused by multiple factors, including genetic and environmental.

A major risk factor for asthma development has been identified as familial history of the disease. The heritability of asthma ranges from 35-70% (Morales & Duffy, 2019). Its severity depends on whether both parents have it and how chronic it is. It is proposed that maternal rates of inheritance contribute significantly more to transmission rates than paternal rates (Liu et al., 2018). Mutations also influence the asthma disease rate; malfunctions with SNPs, histone acetylation or HLA haplotypes contribute to asthma development (Choi et al., 2021). Environmental risk factors include, but are not limited to, air pollution, tobacco smoke, respiratory viral infection, and microbial exposures. General allergies and obesity can contribute to asthma severity. As such, asthma is characterized as a complex disease established by genetic, epigenetic, and/or environmental factors.

Regardless of what trigger type, asthma response is distinguished by inflammation, mucus buildup and bronchoconstriction (CDC, 2017). People afflicted with asthma typically display multiple symptoms simultaneously. These include shortness of breath, dry short coughs, chest tightness and pain, and wheezing (CDC, 2017). More chronic cases of asthma result in sleep disturbance and a drop in the peak flow meter. Symptoms for the onset of an asthma attack include failure of quick-relief medicines, immobilizing chest tightness, severe breath shortness and hunched shoulders. The failure to treat these asthma symptoms results in increased disease severity and pain.

Yet treatment cannot be obtained without at least understanding some of the cellular mechanisms behind asthma. There are several classifications of asthma. Type 1 asthma, known as "brittle asthma," is difficulties in daily breathing, whereas type 2, which is the primary focus in most research, is characterized by excessive T helper cells. There is also allergic or nonallergic asthma. Regardless, each case of asthma induces pathological changes in the cells and their functions. Several mechanisms to examine as potential cellular targets are the role of the homeobox homolog, Six1 promoting exacerbations and miRNAs mediating effects on Six1. As well as the upregulation and/or dysregulation of interleukins and estrogen receptors have also been recently observed when determining effective biological treatments.

Our understanding of asthma and its treatment has come a long way. In the 1500s, asthma was treated with tobacco; the reasoning was to induce coughing and mucus expectoration (Asthma & Allergy, 2018). As we know now, tobacco is a carcinogen and a lung irritant, thus contributing to asthma. People now have inhalers and pills to control their asthma. However, greater advancements in treatment are needed to effectively treat and reduce asthma. Currently, there are two types of asthma treatment: short-term and long-term. Short-term asthma caused by allergies or respiratory functions are treated with oral and intravenous corticosteroids; these include prednisone and methylprednisolone (Mayo Clinic, 2022). Depending on the length of asthma with respiratory infection, an inhaler or nebulizer is used to treat it.

Long-term asthma requires everyday treatment. To mediate long-term asthma, patients are requested to avoid any additional triggers. They are also provided with inhaled corticosteroids (ICS), most commonly fluticasone propionate. Oral medications are prescribed to help relieve symptoms. People with moderate-severe asthma use combined inhalers, such as budesonide-formoterol (Symbicort), and may need both corticosteroids and combination inhalers to treat asthma (Mayo Clinic, 2022). Recent treatments have included the development of an Asthma Action Plan. These plans include a chart with a description of symptoms under 3 levels. For however many symptoms or intensity is being experienced, patients are guided by the chart to what action they must perform. At level three asthma, patients are directed to the emergency room for treatment. These treatment methods are an improvement from the past, but they mainly work to alleviate asthma symptoms. But none are fully effective at opening or maintaining the airway clearance.

Treatment is generally able to subdue asthma symptoms. However, patients with severe asthma cannot be treated normally. These patients are categorized by an abnormal lack of control while on high dose ICS plus a second medication (inhaled or oral) (Busse, 2019). For them, there are few options. Consequently, doctors choose to prescribe Theophylline, a daily pill that relaxes muscles to keep our airways open. Unfortunately, this medication is outdated and requires daily blood tests (Mayo Clinic, 2022); alternative asthma medications are suggested. There remains a need for innovative treatment methods that are precise to the development of asthma and that patient. Without this, asthma diagnoses and deaths may continue to rise. Once the genetics of asthma pathogenesis are well understood, new individualized therapies and prevention methods can develop.

The Cellular Mechanisms of Asthma

In simplistic terms, asthma is characterized by immune dysregulation. However, there are several mechanisms examined as proponents to asthma disease and diagnosis. The homeodomain transcription factor sine coulis homeobox homolog (Six1) is one mechanism under review. Further, Six1 is located on the human chromosome 14q23 and was originally identified as a TGF-b-inducible gene. This was because it is generated by the nonhematopoietic cells stimulated from TGF-b, TNF-a, and particular interleukins (Wang et al., 2021). Six1 is released from mesenchymal cells to target immune cells and promote proinflammatory cytokines and chemokines. Normally, Six1 is expressed at very low levels in normal adult tissue, but its contribution to carcinogenesis has been shown in several diseases such as lung cancer and pulmonary fibrosis (Wang et al., 2021). Increased levels of Six1 are naturally present during embryonic development as it plays crucial roles in cell proliferation, differentiation, survival, and lung development (Wang et al., 2021). Upregulated expression of Six1 appears to indicate proinflammatory effects that negatively affect a patient.

Yang and colleagues (2016) previously confirmed the presence

of Six1 in asthma and that it played a key role in pathogenesis. In that study researchers utilized an ovalbumin-induced mice model and analyzed findings through immunohistochemistry and western blotting to evaluate the expression level of Six1. Further, it was revealed that Six1 disrupted regular lung tissue and was significantly overexpressed in asthma patients when compared to the negative control. Additionally, Six1 effect on various leukocytes was also examined; cell numbers for each immune cell were highly increased in the upregulated Six1 expressing OVA mice, compared to the control. Knockdown of Six1 showed decreased numbers. This study confirmed the presence of Six1 in asthma and its effects on several immune cells. However, the exact relationship between Six1 and airway remodeling is still being investigated.

Airway remodeling is a main mechanism involved in irregular lung and bronchus asthma effects. Specifically, it is a pathological change in severe asthma and constitutes extracellular and cellular changes in the airways. This prognosis has contributed to fibroblast activation, airway smooth muscle (ASM) proliferation and epithelial cell apoptosis (Hough et al., 2020). One of its main components, epithelial-mesenchymal transition (EMT), may have direct linkage to Six1 and fibrosis. Furthermore, Wang et al. (2021) discovered Six1 promotes EMT in bronchial epithelial cells. Results indicated that Six1 promoted EMT through the TGFB1/Smad pathway. This pathway is important in mediating fibrosis. Smad is a downstream regulator and when exposed to a ligand, Smad protein nuclear localization is stimulated and regulates targeted gene expression. For asthma patients, activation of this pathway promotes fibrosis and ultimately stimulates airway remodeling as a response.

Within this study, in cells overexpressing Six1, researchers were able to determine through rt-PCR and western blotting that both TGFB1 and Smad were significantly overexpressed compared to the negative control. However, when Six1 was inhibited, TGFB1 and Smad protein expression decreased. This study indicated that Six1 expression contributes to airway remodeling. More specifically, the relationship between Six1 and TGFB1/Smad revealed extensive fibrosis and EMT enhancement. Six1 overexpression in asthma promotes negative effects on cell morphology, pathology, and expression. Potential effective treatments should focus on inhibiting Six1 in asthma patients.

Asthma is also associated with increased IgE production within the lungs. Therefore, researchers have also aimed to focus on more biologic components to treat, such as inflammatory response. Inflammation is a key characteristic of asthma and is triggered by interleukin-4 (IL-4) and IL-13. Mediated by receptors, IL-4 and IL-13 play a pivotal role in severe asthma. IL-4 is heavily involved in T helper 2 (Th2) lymphocyte cell differentiation, while IL-13 works alongside IL-4 to promote IgE synthesis, fibroblast proliferation and elicit ASM response (Pelaia et al., 2022). Both interleukins share common signaling pathways as when selective parts of IL-4 are blocked, IL-13 has potential to also be blocked. In severe asthma, these cytokines primarily work to amplify bronchial inflammation, persistence, and remodeling. When overexpressed, their presence is indicated by heightened IgE levels in lung samples.

The interleukin-4 pathway is mediated by its receptor IL-4R signaling system. This system includes two receptors, type 1, and type 2. However, type 2 IL-4R is more associated with interleukin 13 as it comprises an IL-4Ra chain paired with IL-13Ra1 and can be activated by IL-13. Type 1 IL-4R cannot be activated by interleukin 13 (Karo et al., 2018). In general, initiation of the signal transduction requires receptor-associated kinase phosphorylation. IL-4 does not naturally contain endogenous kinase activity and so in asthma, its activation is due to Jak2 and/or tyrosine kinase 2 (Tyk2) (Ladjemi et al., 2018). When engaged with IL-4 or IL-13, Jak phosphorylates and dimerizes STAT6. Subsequently, translocation of STAT6 occurs and induces the transcription of IL-4 and IL-13 thus promoting IL4 and/or IL-13 responses.

In severe asthma, this mediated response induces differentiation of B cells into IgE secreting plasma cells and raises the level of IgE antibodies in the blood. With repeated exposure and secretion, IgE cross-linking occurs on mast cells and induces histamine secretion and chemokines that cause inflammatory cells, importantly eosinophils, transmigration (Karo et al., 2018). The increased IL-4 and IL-13 levels subsequently result in epithelial cell hyperplasia, tissue/airway remodeling and mucus hypersecretion.

The effects of IL-4 and IL-13 secretion can be significant in asthma patients. Beckert and colleagues (2020) determined that in blood, IL-13 significantly increased the percent of eosinophils after 72 hours, though its presence was notably detected only after 24. After IL-13 installation faded, this percentage decreased (168-hour mark). To emphasize the role of IL-4 and IL-13 compared to other cytokines or factors, researchers noted that only IL-4 and –13 were able to induce early-on eosinophil transmigration into the airway for BAL. Researchers emphasize that in two scenarios, IL-5 failed to induce early time point transmigration, indicating that its role in asthma is not as prevalent. Researchers from this study also observed the combined effects of cytokines. As mentioned, for asthma, IL-4 and IL-13 share many similar signaling pathways even though sequence wise, they are only 25% related. Interestingly, IL-4 and IL-13 in combination showed no eosinophil number increase compared to individual measurements. However, all three cytokines IL-4, -13, and –5, when combined resulted in increased total and relative eosinophil numbers (Beckert et al., 2020).

To further verify the role of IL-4 and IL-13 in asthma, the same researchers noted several knockout tests and saw that knockout of IL-4 did not affect IL-13 related lung eosinophilia. However, the knockout of IL-13 affected downstream signaling components such as the IL-4a chain and STAT6 and affected goblet cell metaplasia as well as eosinophilia. Interleukin 5 did result in a higher eosinophil concentration in the bone marrow, but not so much in the blood. Taken together, this study demonstrated the effects of type 2 cytokines, most importantly, IL-4 and IL-13 on the disease-driving mechanisms of asthma. Further, this study indicated IL-13 may play a more dominant role in regulating migration of eosinophils into the airways than once thought of, compared to IL-4. From this perspective, IL-13 appears to have modulatory functions during sensitization with stronger effects than those of IL-4.

In understanding asthma and its inflammatory responses, the evaluation of interleukins has led way to more recent understandings of steroid resistant asthma, also known as severe asthma. While steroid resistant asthma patients account for only 5-10% of the asthma population, the mechanisms behind it are still unclear (Wadhwa, 2019). Several studies have supported the idea that intense immune dysregulation among interleukins plays a role in steroid insensitivity. Further, Wang et al. (2021) reported that interleukin –4 and –13 expression by basophils, CD8+ memory cells and group 2 innate lymphoid cells are largely steroid resistant.

Innate lymphoid cells (ILCs), which produce relatively high quantities of IL-4 and –13, have been implicated in the pathogenesis of steroid resistant asthma (Liu et al., 2018). To understand the activation behind these intracellular molecular networks, ILC2 and CD8+ memory T cells were examined under a gene set enrichment analysis by Wang and colleagues (2021). In this study, they determined the canonical pathways and upstream regulators of both variables were substantially upregulated in asthma. Resistance in the T cells was most likely due to an upstream activator of ERK1/2 known as MEK1 which promotes steroid resistance by phosphorylating unliganded glucocorticoid receptor (GR), ultimately sequestering it in the cytosol (Liu et al., 2018). It was further noted that activation of the GR inhibited expression of mRNA for STAT and JAK upstream activators; STAT knockdown reserved steroid resistance.

Wang and colleagues (2021) especially reiterated the role of IL-13 as a key cytokine in driving the pathogenesis of asthma and steroid resistance to glucocorticoids. They found that when IL-13 was neutralized, steroid-resistant airway hyperresponsiveness decreased. Furthermore, anti-IL-13 suppressed eosinophil, neutrophil and lymphocyte infiltration. It is suggested that IL-13 is necessary for lipopolysaccharide regulated steroid resistant airway inflammation and hyperresponsiveness.

The Role of Viruses in Asthma

Respiratory virus infections are a key contributor to asthma exacerbations. RV, Rhinovirus, is the most prevalent human respiratory virus (Kim, 2018). RV replicated in epithelial cells and is typically

associated with acute exacerbations. It was once thought of only affecting the upper airways; however, it affects both levels of the airway and is more predisposed in asthmatic patients because of damaged epithelium and airway obstruction. RV infection in asthma induces higher intracellular adhesion molecule-1 (ICAM-1) expression, deficient Th1 response, increased lysis, disrupted epithelial barrier and enhanced neutrophil recruitment (Kim, 2018). All of which promote production of interleukins and TNF-a. In negatively affecting these variables, airway eosinophils are increased, subsequently causing fibrosis and airway hyperresponsiveness/remodeling. The second most common virus, respiratory syncytial virus (RSV), has just as detrimental effects on asthmatic patients. Once infected with RSV, airway epithelial cells respond by releasing type I IFNS and a variety of interleukins. Under some conditions, RSV infection could promote higher Th2 response compared to Th1. This was noted in a study that found IFN-y and TNF-a levels were significantly lower in RSV-induced asthma, compared to influenza (Matsuse et al., 2013).

Our understanding of viruses in asthmatic patients is scarce, but increased levels of eosinophils may indicate a protective role in the human immune system. Further, Pineros et al. (2018) found that in healthy patient blood samples infected with DiD, most eosinophils were DiD-positive at 120-minutes. Through fluorescence intensity, diffuse staining increased over time, indicating the number of viral particles (DiD) captured by eosinophils also increased. In turn, this effectively reduced infectivity. This finding was the opposite in asthma patients. Furthermore, blood eosinophils from asthma patients had reduced DiD- labeled RSV uptake. Upon closer examination, in severe asthmatics with high doses of corticosteroids, there was a larger capacity reduction by eosinophils to bind to the virus compared with healthy patients (Pineros et al., 2018); for some, this value was as low as 20% binding ability. Less binding affinity has also resulted in reduced ability to effectively inactivate viruses in asthma patients, compared to healthy control.

Interestingly, eosinophils in healthy patients displayed beneficial immune properties. Whereas in asthma patients they tend to exert cytotoxic properties exacerbate their condition. The aforementioned study revealed that eosinophils bound to viruses became masticated and altered cell surface molecules to release pro-inflammatory cytokines to deactivate the virus. It is speculated for asthma patients that the number and activation state control an eosinophil antiviral response. However, eosinophils in asthma patients may already be persistently activated in circulation which may explain reduced capacity to bind to viruses. It was suggested that the medication severe asthma patients use could hinder the antiviral effects of eosinophils. Specifically, these researchers speculated that tapering anti-IL-5 or other anti-interleukins to limit eosinophils in asthma patients may be a better treatment option compared to complete eradication.

Gender in Asthma

Asthma diagnoses range in age and gender. Generally, asthma diagnoses are more prevalent among boys than girls during childhood, whereas after puberty, the opposite occurs, and more females are diagnosed as adults. This suggests that hormones may play a role in Asthma diagnoses and exacerbations. However, the mechanism behind gender differences is still being determined.

Cytokines have played a critical role in asthma and in 2013, a study performed by Takeda and colleagues measured the concentrations of T-helper (Th1 & Th2) cytokines between OVA-induced women and men using enzyme-linked immunosorbent assay. In this assay, they discovered IL-13 and TGF-B were significantly increased in the BALF of OVA females compared to OVA males. Subsequently this revealed significantly elevated IgE levels and more specifically serum IgA, two indicators of asthma exacerbations, were also raised in OVA-induced females. Since airway remodeling is in part orchestrated by IL-13, researchers determined asthmatic females were more susceptible to airway remodeling and goblet cell hyperplasia. Additionally, the presence of increased TGF-B is most often associated with increased eosinophil infiltration in lung tissue, a contributor to airway remodeling. In this study, researchers saw the number of eosinophils were raised in OVA female compared with OVA males. Furthermore, researchers in this study demonstrated one of the first understandings of the role of gender in asthma and specifically discovered that these variables, increased in asthmatic women could be correlated with increased tissue fibrosis, and thickness of airway smooth muscle and mucosa. However, the exact mechanisms behind the potential role of hormones in affecting interleukin or TGF concentrations is still being determined.

A more recent study by Ambhore and colleagues (2019) has shed some light onto the potential mechanisms behind these gender differences. Researchers in this study evaluated several key characteristics of asthma and the effects of estrogen. In general, estrogen has been shown to influence the immune system through estrogen receptors, ER-a and ER-b. Regarding asthma, it was proposed that ER-b plays a more significant role in mediating inflammatory responses. When determining the effects of estrogen receptors, Ambhore et al. examined airway resistance, pulmonary compliance and elastance. In all models, it was reported that women were negatively affected. More specifically, ER-b was most expressed in asthmatic mice. Women showed significant ER-b expression that was more than double the original expression (from 1AU to 3AU). OVX female mice showed under MA conditions, ER-a expression was not elevated as much (compared to male and female model); it also was less expressed compared to ER-b. Estrogen beta was further examined to evaluate whether it could remedy asthma exacerbations. WAY, ER beta agonist, was able to reverse these effects and showed significant ER beta expression in MA mice. WAY also showed better effects at reducing ER-a overexpression in female mice, compared to its regular agonist PPT.

As supported by other studies, Ambhore et al. found elevated levels of eosinophils in asthmatic mice, but especially asthmatic female mice. Researchers also examined how the activation of estrogen receptors affected airway remodeling and hyperresponsiveness. ER-b agonist showed the most promising effects for returning AHR/airway modeling factors back to normal/ control. Interestingly, ER-a activation upregulated eosinophil infiltration which worsened AHR and ASM. ER-b activation also increased airway compliance and reduced elastance. ER-a agonist showed similar benefits for asthmatic mice. However, ER-a activation did not improve respiratory function as well as ER-b activation.

With higher asthma prevalence in women, researchers have started to question whether there could be a maternal influence on asthma risk in offspring. Poorly controlled asthma during pregnancy can affect fetal development negatively and has been linked to fetal growth restrictions. Not many studies have revealed the relationship, but one population-based cohort study, performed by Liu and colleagues (2017), found that children born to asthmatic mothers had higher mild, moderate, and severe asthma prevalence compared to non-asthmatic mothers. Studies have already shown that there is a genetic predisposition, but whether asthma severity during pregnancy affects this rate is uncertain. In response to this, Liu et al. discovered a higher prevalence of early-onset persistent asthma and early-onset transient asthma in asthmatic mothers with controlled asthma compared to the opposite group. It is possible that uncontrolled asthma may amplify effects of maternal asthma on fetal hypoxia, as another study reported that children with onset persistent or transient asthma had reduced lung function.

More recent studies have been researching whether hormone replacement therapy (HRT) is associated with new asthma cases. To deepen our understanding, Hansen et al. (2021) sought to investigate whether HRT alters incidence of asthma. To do this, they mainly examined the hazard ratio, which is a measure of how often an event occurs in one group compared to another overtime. Overall, they observed that women who actively receiving hormone replacement therapy has an adjusted hazard ratio of 1.63 of new asthma development compared to women not receiving the HRT. Their classification of asthma was defined by use of inhaled corticosteroids needed after/during HRT, not prior to. Researchers then ranked their analysis into active HRT subtypes and discovered that estrogen monotherapy combined with progesterone increased hazard ratio of new asthma in women. Whereas progesterone monotherapy decreased the hazard ratio of new asthma. This study measured the association between HRT and increased new asthma; however, findings need more clarity before a correlation or causation relationship is ascertained. Future studies should focus more specifically on the individual effects estrogen implantation has on certain asthma exacerbations.

Regardless, this study put forth a complex relationship between menopause, asthma and hormone replacement therapy that showed HRT increased new asthma HR, and its termination was correlated with discontinuation of asthma treatment.

Treatments in Asthma

Advancements in treatment methods are important to provide efficient, effective management/control for all types of asthma. Currently, corticosteroids, which decrease airway inflammation are the backbone of asthma treatment. Therapies with microRNAs are now being considered. MicroRNAs (miRNAs) are endogenous non-coding RNA molecules. They can regulate expression by binding to complementary sequences in the 3' UTR and lead to mRNA translational termination or degradation (Yang et al., 2020). Several miRNAs, such as miR-488-5p, miR-106b-5p, and miR-203a-3p have been reported regulators in asthmatic pathways.

Yang and colleagues (2018) reported miR-488-5p expression was significantly decreased in lung tissues of asthmatic mice. Consequently, Six1 expression was remarkably increased in asthmatic and TGF-B1 stimulated cells. To investigate whether there was a relationship between these variables, researchers in this study transfected miR-488-5p or its inhibitor into 16HBE cells. As expected, the level of expression increased in mice transfected with the miRNA mimic. More specifically, it was noted that the introduction of miR-488-5p significantly suppressed TGF-B1 induced fibronectin and collagen IV expression. Overexpression of this miRNA inhibited asthma exacerbations. Similar results were examined when Six1 was knocked down, in which silencing Six1 reduced TGF-B1 stimulated fibronectin expression. MiR-488-5p knockdown increased exacerbations. This study determined that the upregulation of miR-488-5p sufficiently inhibited TGF-B1 stimulated epithelial-mesenchymal transition (EMT). Additionally, overexpression of miR-488-5p reduced Six1, which in turn, when silenced, effectively prevents TGF-B1 fibrosis and airway remodeling.

Another study performed by Liu et al. (2021) focused on the role of miR-106b-5p. Similarly, this study saw that expression levels of miR-106b were significantly downregulated in asthmatic and TGF-B1 stimulated mice, meanwhile Six1 was upregulated. However, researchers in this study discovered miR-106b-5p works to inhibit Six1 by targeting the gene transcription factor E2F1. Inhibition of E2F1 expression significantly decreased TGF-B1 and Six1 stimulated increases in vimentin and fibrosis. To verify whether upregulation of miR-106b-5p efficiently treats asthma symptoms. Liu et al. transfected miR-106b-5p mimic and anti miR-106b-5p with pGL3-451 or a mutated version of that of the E2F1 binding sites. Luciferase assay revealed miR-106b-5p overexpression inhibited pGL3-451 activity. The knockdown of this miRNA had the opposite effect. Upon closer examination, the level of E2F1 binding to SIX1 promoter was decreased in miR-106b transfected cells, but increased when antimiR-106b was transfected. Overall, this study revealed the potential therapeutic role that miR-106b-5p may negatively regulate SIX1 expression via E2F1 inhibition.

MiRNAs have the potential for restoration-mediated effects on asthma symptoms. Fan & Jian (2020) had findings that aligned with the previous two studies, but with miR-203a-3p. In all cases, overexpression of miRNA has led to reduced asthma exacerbations. However, studies are beginning to show the individual roles each miRNA plays in asthma. For instance, miR-488-5p targeted Six1 through TGF-B1 and miR-106b-5p targeted Six1 through E2F1. MiR-203a-3p regulates TGF-B1 induced EMT by regulating the Smad3 pathway. In Fan and Jian's study they found that SIX1 levels were inversely correlated with miR-203a-3- expression in asthma samples. To mediate these levels, researchers saw that TGF-B1 treated cells had increased SMad3 protein expression which led to the phosphorylation of kinase C in TGF-B1. Subsequently, this induced fibrosis and EMT. However, miR-203a-3p re-expression reversed these effects. This study further supports the theory that overexpression of certain miRNAs could serve as therapeutic agents in Six1/TGF-B1 induced asthma exacerbations.

For asthma patients classified as steroid resistant, most treatments are rendered ineffective. Current treatments have focused on more biologic approaches; for example, Dupilumab, which is a human immune-derived monoclonal antibody that works to inhibit IL-4 and IL-13 signaling. Previous therapies have focused on inhibiting interleukins; however, these approaches were not that effective. For instance, pascolizumab inhibits IL-4 and its receptor from interacting, which promotes repression of Th2 cell differentiation, eosinophilia and IgE up-regulation (Ricciardolo et al., 2021). Despite this, IL-13 still had the ability to interact and induce IL-4R responses and so was not continued in a phase 3 study. The issue that arises in asthma therapy for severe patients is inhibition of both interleukins that play a key role in asthma pathogenesis. Fortunately, Dupilumab inhibits IL-4R induced by IL-4 or IL-13. In doing this, it down regulates Th2 inflammation and suppresses eosinophilia as well as reduces IgE production.

Using a statistical software, QUEST, Corren and colleagues (2020) studied the effects of dupilumab treatment. In this study, dupilumab significantly reduced severe asthma exacerbations and improved FEV1 scores, a measure of forced expiratory volume in one second. Specifically, at week 12, treatment with 200 and 300-mg of dupilumab improved prebronchodilator FEV compared to the placebo group. This was identified by the magnitude of improvement being equal or greater than patients with eosinophil levels at 140 and 300 cell/uL. Consistent with the mechanisms behind Dupilumab, researchers also discovered this therapy significantly reduced serum total IgE in asthmatic patients.

Yet few patients can financially access dupilumab treatment and so cases of severe, steroid-resistant, asthma continue to persist. Wang et al. (2018) researched an alternative approach by targeting the phosphorylation site of myristoylated alanine-rich C kinase (MARCKS). This substrate facilitates mucus production and migration and is highly expressed in inflammatory cells in asthmatic patients. Following the treatment with 2.3 mg/kg of MPS peptide, all responding cytokines were suppressed. In comparison with a current treatment, dexamethasone (Dexa), MPS peptide reduced leukocyte levels, collagen deposition, and airway hyperresponsiveness. Dexa on the other hand did not alleviate symptoms. Researchers then determined what properties of the MPS peptide made it effective in inhibiting MARCKS phosphorylation. To which they discovered through Asp or Ala mutations that Ser residues can suppress increased phosphorylated PKC expression in the lungs of asthmatic mice. Scientists continue to struggle to effectively prevent or reduce severe asthma exacerbations in patients with steroid-resistance. However, this study offered a potential target treatment that works to inhibit neutrophilic inflammation and relieve symptoms through a peptide designed to specifically target the phosphorylation site of MARCKS.

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