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Nrf2 Signaling and Its Role in Redox Homeostasis in Sickle Cell Anemia

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Abstract

Sickle Cell Anemia (SCA) is characterized by chronic oxidative stress and redox imbalance due to the polymerization of hemoglobin S (HbS), leading to hemolysis, vaso-occlusion, and organ damage. The nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway plays a critical role in cellular defense against oxidative stress by regulating the expression of antioxidant and cytoprotective genes. This review examines the role of Nrf2 in maintaining redox homeostasis in SCA, highlighting its mechanisms of activation and its potential as a therapeutic target. The Nrf2 pathway is activated in response to oxidative stress when Nrf2 dissociates from its inhibitor Keap1, translocates to the nucleus, and binds to antioxidant response elements (ARE) in the promoter regions of target genes. This activation results in the upregulation of genes encoding antioxidant proteins, such as glutathione S-transferase, NAD(P)H oxidoreductase 1 (NQO1), and heme oxygenase-1 (HO-1), which are crucial for neutralizing reactive oxygen species (ROS) and maintaining cellular redox balance. In SCA, Nrf2 activation can mitigate oxidative damage, reduce hemolysis, and alleviate vaso-occlusive crises and inflammation. Therapeutic strategies that enhance Nrf2 signaling offer significant potential in the treatment of SCA. Pharmacological activators of Nrf2, such as bardoxolone methyl and dimethyl fumarate, have shown promise in preclinical and clinical studies for other oxidative stress-related conditions and may be beneficial for SCA patients. Additionally, genetic approaches like gene therapy or CRISPR/Cas9-mediated gene editing to enhance Nrf2 activity could provide long-term protection against oxidative stress.

Keywords: *Nrf2 Signaling, Redox Homeostasis, Sickle Cell Anemia, Oxidative Stress, Antioxidant Response*

Introduction

Sickle Cell Anemia (SCA) is a hereditary hemoglobinopathy caused by a single nucleotide mutation in the β-globin gene, leading to the production of hemoglobin S (HbS) instead of normal hemoglobin A (HbA). This mutation results in the substitution of glutamic acid with valine at the sixth position of the β-globin chain, which promotes the polymerization of HbS under low oxygen conditions. The polymerization causes red blood cells (RBCs) to adopt a sickle-shaped morphology, impairing their deformability and leading to vaso-occlusive crises, hemolysis, and chronic organ damage. A significant aspect of SCA pathology is oxidative stress, which arises from the imbalance between the production of reactive oxygen species (ROS) and the body's

ability to neutralize these harmful molecules through antioxidant defenses. Oxidative stress in SCA is exacerbated by the breakdown of sickled RBCs, which releases free hemoglobin and heme into the bloodstream. Free hemoglobin can be oxidized to methemoglobin, and free heme can catalyze the production of ROS through Fenton-type reactions. The resulting ROS cause widespread cellular damage, affecting not only RBCs but also endothelial cells, leading to inflammation and contributing to the disease's chronic complications. Thus, understanding and targeting oxidative stress is central to managing and potentially alleviating the symptoms of SCA. The nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway is a fundamental regulator of cellular antioxidant responses and redox homeostasis. Under normal conditions, Nrf2 is kept in the cytoplasm in a complex with Kelch-like ECH-associated protein 1 (Keap1), which facilitates its ubiquitination and proteasomal degradation. However, in response to oxidative stress or electrophilic agents, Nrf2 is released from Keap1 and translocates to the nucleus. Once in the nucleus, Nrf2 binds to antioxidant response elements (AREs) in the promoter regions of target genes, driving the transcription of genes involved in antioxidant defense and detoxification processes.[1-10]

Key genes activated by Nrf2 include those encoding for heme oxygenase-1 (HO-1), which breaks down heme into biliverdin, carbon monoxide, and free iron; glutathione S-transferase (GST), which facilitates the conjugation of ROS with glutathione for detoxification; and NAD(P)H oxidoreductase 1 (NQO1), which reduces quinones and protects cells from oxidative damage. By upregulating these and other antioxidant genes, Nrf2 helps maintain redox balance, protect cells from oxidative damage, and promote cellular survival under stress conditions. In SCA, the Nrf2 pathway is of particular interest due to its potential to counteract the oxidative stress induced by sickling and hemolysis. Studies have shown that Nrf2 activity is elevated in various models of oxidative stress and can be harnessed to mitigate cellular damage. In SCA, Nrf2 activation can lead to increased expression of antioxidant enzymes and protective proteins, thereby reducing the oxidative damage associated with sickle cell pathology. For instance, Nrf2-mediated upregulation of HO-1 can reduce the oxidative burden from free heme released during hemolysis, while increased levels of GST and NQO1 can help neutralize ROS generated from the sickling process. Research has also demonstrated that pharmacological activation of Nrf2 using small molecules such as bardoxolone methyl and dimethyl fumarate can alleviate oxidative stress and improve outcomes in other oxidative stress-related diseases. These findings suggest that similar therapeutic strategies could be applied to SCA to enhance Nrf2 activity and mitigate disease symptoms. By promoting Nrf2 activation, these drugs could help reduce hemolysis, alleviate vaso-occlusive events, and improve overall patient health.[11-20]

Nrf2 is integral to the cellular antioxidant defense system, which consists of various mechanisms designed to neutralize ROS and repair oxidative damage. Antioxidant enzymes regulated by Nrf2, such as HO-1, GST, and NQO1, are essential for detoxifying ROS and protecting cells from oxidative damage. HO-1, for example, breaks down heme into biliverdin, a potent antioxidant that can reduce ROS levels and limit inflammation. GST enzymes conjugate ROS with glutathione, facilitating their excretion from the cell, while NQO1 protects cells from oxidative stress by reducing quinones and preventing ROS generation. In addition to these direct antioxidant effects, Nrf2 activation also upregulates genes involved in the synthesis and regeneration of glutathione, a

key intracellular antioxidant. Glutathione is crucial for maintaining cellular redox balance and mitigating oxidative damage. The ability of Nrf2 to coordinate these diverse antioxidant responses underscores its importance as a therapeutic target for diseases characterized by excessive oxidative stress, including SCA. The therapeutic potential of Nrf2 activation in SCA lies in its ability to counteract oxidative stress and its associated effects. By enhancing the expression of antioxidant and cytoprotective genes, Nrf2 activators could potentially reduce the severity of vaso-occlusive crises, alleviate hemolysis, and diminish the inflammatory responses seen in SCA. For example, preclinical studies have shown that Nrf2 activators can reduce oxidative damage, improve RBC survival, and mitigate inflammation in animal models of SCA. These findings provide a strong rationale for exploring Nrf2 activation as a therapeutic approach for managing SCA.[21-30]

Pharmacological Nrf2 activators, such as bardoxolone methyl and dimethyl fumarate, have demonstrated efficacy in clinical trials for other diseases by modulating oxidative stress and inflammation. Their potential benefits for SCA are being investigated, with the hope that these agents can improve patient outcomes by targeting the root causes of oxidative damage and inflammation in SCA. The development of Nrf2 activators as therapeutics for SCA represents a novel approach that could complement existing treatments and offer new avenues for disease management. In addition to pharmacological approaches, genetic strategies to enhance Nrf2 activity offer exciting prospects for SCA therapy. Advances in gene therapy, including CRISPR/Cas9-mediated gene editing, allow for targeted manipulation of the Nrf2 pathway to achieve therapeutic effects. For example, strategies to increase the expression of Nrf2 or enhance its activity could provide a long-term solution to managing oxidative stress in SCA. Gene therapy approaches might also involve the introduction of Nrf2-targeted gene constructs or the modification of endogenous Nrf2 genes to enhance their function. Such approaches could offer sustained protection against oxidative stress and reduce the frequency of vaso-occlusive crises. The exploration of these genetic strategies underscores the potential for innovative treatments that go beyond conventional pharmacological therapies.[31-35]

The Nrf2 Signaling Pathway

The nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway is a critical component of the cellular defense system against oxidative stress. This pathway governs the expression of a range of antioxidant and cytoprotective genes that help maintain cellular redox homeostasis. To fully appreciate the role of Nrf2, it is essential to explore its regulatory mechanisms, activation processes, and downstream effects on cellular antioxidant defenses. Nrf2 is a transcription factor that resides in the cytoplasm under normal conditions in a complex with its negative regulator, Kelch-like ECH-associated protein 1 (Keap1). In this complex, Keap1 functions as a substrate adaptor for the Cullin 3-based E3 ubiquitin ligase complex, which tags Nrf2 for degradation via the proteasome. This system ensures that Nrf2 levels remain low under non-stressful conditions, preventing unnecessary activation of antioxidant responses. Under conditions of oxidative stress, however, the Nrf2-Keap1 interaction is disrupted. Oxidants or electrophiles modify specific cysteine residues on Keap1, leading to a conformational change that inhibits Keap1's ability to promote Nrf2 degradation. As a result, Nrf2 accumulates in the cytoplasm and translocates to the nucleus, where it exerts its protective effects by initiating the transcription of antioxidant genes.

The activation of Nrf2 is a finely tuned process that involves several key steps. First, oxidative or electrophilic stress induces modifications on the cysteine residues of Keap1. These modifications prevent Keap1 from recognizing Nrf2 for ubiquitination, leading to an increase in Nrf2 levels. Once liberated from Keap1, Nrf2 translocates to the nucleus through the nuclear import pathway, aided by the nuclear localization signal present in the Nrf2 protein.[36-40]

In the nucleus, Nrf2 binds to antioxidant response elements (AREs) located in the promoter regions of target genes. This interaction facilitates the recruitment of co-activators, such as CREB-binding protein (CBP) and p300, which enhance the transcription of genes encoding for antioxidant enzymes and phase II detoxification enzymes. These enzymes play crucial roles in mitigating oxidative damage and promoting cellular survival under stress conditions. The Nrf2 pathway regulates a diverse array of target genes involved in antioxidant defense and cellular protection. One of the primary target genes is heme oxygenase-1 (HO-1), which catalyzes the breakdown of heme into biliverdin, carbon monoxide, and free iron. Biliverdin and carbon monoxide have well-documented antioxidant and anti-inflammatory properties, while free iron is sequestered by ferritin to prevent oxidative damage. Another important target gene is glutathione S-transferase (GST), which plays a key role in the detoxification of ROS and electrophiles. GST enzymes conjugate ROS with glutathione, facilitating their removal from the cell. NAD(P)H oxidoreductase 1 (NQO1) is another Nrf2 target gene that helps reduce quinones to less harmful forms, further contributing to cellular defense against oxidative stress. Additionally, Nrf2 regulates genes involved in glutathione synthesis and regeneration, such as glutamate-cysteine ligase (GCL). GCL is responsible for the synthesis of glutathione, one of the cell's most potent antioxidants. By upregulating these and other protective genes, Nrf2 ensures a robust antioxidant response to counteract oxidative damage.[41-45]

The Nrf2 signaling pathway is crucial in protecting against various diseases associated with oxidative stress. Its activation has been linked to protective effects in conditions such as cardiovascular diseases, neurodegenerative disorders, and cancer. In Sickle Cell Anemia (SCA), Nrf2's role is particularly significant due to the chronic oxidative stress experienced by patients. In SCA, the excessive oxidative stress from sickle cell hemolysis and vaso-occlusive crises leads to severe cellular damage and disease complications. By enhancing Nrf2 activity, it is possible to improve the antioxidant defenses of RBCs, reduce oxidative damage, and alleviate symptoms of the disease. This has been demonstrated in preclinical studies where Nrf2 activators reduced oxidative damage and improved disease outcomes in models of SCA. Pharmacological agents that activate Nrf2, such as bardoxolone methyl and dimethyl fumarate, are being explored for their potential to treat SCA. These compounds induce Nrf2 activation through interactions with Keap1, leading to increased expression of antioxidant and cytoprotective genes. Their ability to modulate the Nrf2 pathway offers a promising therapeutic strategy for managing SCA and other diseases characterized by oxidative stress.[46-50]

Nrf2 and Redox Homeostasis in SCA

Sickle Cell Anemia (SCA) is a genetic disorder characterized by the presence of hemoglobin S (HbS), which leads to the formation of sickle-shaped red blood cells (RBCs) under low oxygen

conditions. These sickle-shaped cells cause blockages in blood vessels, leading to pain crises, chronic hemolysis, and severe organ damage. A significant aspect of SCA pathology is the chronic oxidative stress that affects both RBCs and other cell types, contributing to the disease's symptoms and complications. Redox homeostasis, or the balance between oxidative and reductive processes in cells, is crucial for managing this stress and maintaining cellular health. Oxidative stress in SCA is driven by several factors. The sickling of RBCs results in hemolysis, which releases free hemoglobin and heme into the bloodstream. These molecules can catalyze the production of reactive oxygen species (ROS), leading to further cellular damage. Additionally, the repeated cycles of ischemia and reperfusion during vaso-occlusive crises exacerbate oxidative stress, causing damage to RBC membranes, endothelial cells, and various tissues. Consequently, maintaining redox homeostasis is vital for managing oxidative damage and improving patient outcomes in SCA. Nuclear factor erythroid 2-related factor 2 (Nrf2) is a central regulator of cellular redox homeostasis and the antioxidant response. Under normal conditions, Nrf2 is bound to Kelch-like ECH-associated protein 1 (Keap1) in the cytoplasm, where Keap1 targets Nrf2 for degradation via the proteasome. This regulatory mechanism keeps Nrf2 levels low and prevents unnecessary activation of antioxidant responses. However, during oxidative stress, Keap1 undergoes modifications that lead to the stabilization and activation of Nrf2. Upon activation, Nrf2 translocates to the nucleus and binds to antioxidant response elements (AREs) in the promoter regions of various target genes. This binding initiates the transcription of genes encoding for antioxidant enzymes, such as heme oxygenase-1 (HO-1), glutathione S-transferase (GST), and NAD(P)H oxidoreductase 1 (NQO1). These enzymes are instrumental in neutralizing ROS, repairing oxidative damage, and protecting cells from stress-induced injury.[51-55]

Nrf2 Activation and Antioxidant Defense in SCA

In SCA, the activation of Nrf2 is critical for counteracting the oxidative stress caused by sickling and hemolysis. Studies have shown that Nrf2 activation increases the expression of HO-1, which degrades free heme into biliverdin, carbon monoxide, and iron. Biliverdin acts as an antioxidant and reduces oxidative stress, while carbon monoxide has anti-inflammatory properties that can mitigate the inflammatory responses in SCA. Additionally, Nrf2 activation leads to increased levels of GST, which facilitates the detoxification of ROS through conjugation with glutathione. This process is essential for neutralizing the ROS generated from sickle cell hemolysis and reducing oxidative damage to cells. Another Nrf2 target gene, NQO1, plays a role in reducing quinones to less harmful forms, thereby protecting cells from oxidative damage and contributing to overall cellular defense. The upregulation of these antioxidant and detoxification enzymes through Nrf2 not only helps manage oxidative stress but also improves the overall health of RBCs and other cells affected by SCA. By enhancing the cellular antioxidant capacity, Nrf2 activation can alleviate some of the primary symptoms of SCA, such as hemolysis and vaso-occlusive crises.[56-60]

Research indicates that Nrf2 activation is impaired in SCA patients, contributing to the chronic oxidative stress observed in the disease. For instance, studies have demonstrated that the levels of Nrf2 and its downstream antioxidant enzymes are lower in SCA patients compared to healthy individuals. This reduced Nrf2 activity is associated with increased oxidative damage, hemolysis,

and inflammation in SCA. One study found that Nrf2 expression and activity are significantly reduced in RBCs from SCA patients, which correlates with increased levels of oxidative markers and decreased antioxidant capacity. This suggests that the dysfunction of Nrf2 in SCA contributes to the oxidative damage seen in the disease and highlights the potential for therapeutic strategies aimed at restoring Nrf2 function. Given the central role of Nrf2 in regulating redox homeostasis, therapeutic strategies that target the Nrf2 pathway offer promising approaches for managing SCA. Pharmacological agents that activate Nrf2, such as bardoxolone methyl and dimethyl fumarate, have shown potential in preclinical studies and clinical trials for other oxidative stress-related diseases. These compounds activate Nrf2 by modifying Keap1, leading to increased expression of antioxidant and cytoprotective genes. In SCA, these Nrf2 activators could help mitigate oxidative stress by enhancing the expression of HO-1, GST, and NQO1, thus reducing oxidative damage and improving patient outcomes. For example, bardoxolone methyl has been shown to improve renal function and reduce inflammation in patients with chronic kidney disease, and similar effects could be beneficial for SCA patients by reducing oxidative damage and inflammation associated with the disease. Another approach is to explore genetic therapies to enhance Nrf2 activity. Advances in gene therapy technologies, such as CRISPR/Cas9-mediated gene editing, offer the potential to increase Nrf2 expression or enhance its function in SCA patients. These genetic approaches could provide long-term therapeutic benefits by directly targeting the Nrf2 pathway and improving the antioxidant defenses of RBCs and other affected cells.[61-70]

Therapeutic Potential of Targeting Nrf2 in SCA

Sickle Cell Anemia (SCA) is a genetic disorder caused by a mutation in the hemoglobin gene that leads to the production of hemoglobin S (HbS). This hemoglobin variant causes red blood cells (RBCs) to become rigid and sickle-shaped under low oxygen conditions, leading to vaso-occlusive crises, chronic hemolysis, and various complications. A significant aspect of SCA pathology is the chronic oxidative stress that affects RBCs and other tissues, contributing to the disease's severe manifestations. The nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway, a critical regulator of the antioxidant response, presents a promising therapeutic target for managing oxidative stress in SCA. Nrf2 is a transcription factor that regulates the expression of antioxidant and cytoprotective genes. Under basal conditions, Nrf2 is kept in the cytoplasm and marked for degradation by its repressor, Kelch-like ECH-associated protein 1 (Keap1). However, in response to oxidative stress, modifications to Keap1 lead to the stabilization and activation of Nrf2. Activated Nrf2 translocates to the nucleus, where it binds to antioxidant response elements (AREs) in the promoter regions of target genes, driving the expression of various antioxidants and detoxifying enzymes.[71-72]

In SCA, the sickling of RBCs and subsequent hemolysis lead to excessive production of reactive oxygen species (ROS), which causes oxidative damage to cells and tissues. The Nrf2 pathway, by upregulating antioxidant defenses, offers a mechanism to counteract this oxidative damage. Thus, enhancing Nrf2 activity could mitigate oxidative stress and improve the clinical outcomes of SCA patients. Several compounds that activate the Nrf2 pathway have shown potential in preclinical models of SCA. For instance, bardoxolone methyl, a potent Nrf2 activator, has demonstrated protective effects in various disease models by inducing the expression of antioxidant enzymes. In

SCA, bardoxolone methyl has been shown to reduce oxidative stress, decrease hemolysis, and improve RBC function in animal models. These effects are achieved through the activation of Nrf2, leading to increased expression of heme oxygenase-1 (HO-1), glutathione S-transferase (GST), and NAD(P)H oxidoreductase 1 (NQO1), which collectively mitigate oxidative damage and inflammation. Similarly, dimethyl fumarate, another Nrf2 activator, has been investigated for its therapeutic effects in SCA. Dimethyl fumarate is known to activate Nrf2 by modifying cysteine residues on Keap1, which leads to increased expression of antioxidant genes. Preclinical studies have shown that dimethyl fumarate reduces oxidative stress, improves RBC deformability, and decreases the severity of vaso-occlusive crises in SCA models. These findings underscore the potential of Nrf2 activation as a therapeutic strategy for managing SCA.[72-73]

The translation of Nrf2-based therapies from preclinical models to clinical practice is an exciting development in the treatment of SCA. Clinical trials exploring the efficacy of Nrf2 activators, such as bardoxolone methyl and dimethyl fumarate, are underway to assess their safety and effectiveness in human patients. For example, clinical trials have been initiated to evaluate bardoxolone methyl's potential to reduce oxidative stress and improve organ function in SCA patients. Preliminary results from these trials suggest that Nrf2 activation may lead to beneficial outcomes in SCA, including reduced hemolysis and improved clinical symptoms. Another emerging therapy is the use of Nrf2 gene therapy approaches, which aim to enhance Nrf2 expression directly. Techniques such as CRISPR/Cas9-mediated gene editing are being explored to increase the expression of Nrf2 or its downstream targets in SCA. These innovative approaches hold the promise of long-term therapeutic benefits by directly modifying the genetic basis of the Nrf2 pathway, offering a novel method for managing oxidative stress in SCA. Targeting the Nrf2 pathway offers several potential benefits for the management of SCA. By enhancing the cellular antioxidant defenses, Nrf2 activators can reduce oxidative stress, which is a central feature of SCA pathology. This reduction in oxidative stress may lead to decreased hemolysis, improved RBC function, and reduced incidence of vaso-occlusive crises. Additionally, Nrf2 activation may also have anti-inflammatory effects, further contributing to the management of SCA symptoms. Furthermore, Nrf2-based therapies have the potential to address multiple aspects of SCA simultaneously. For example, by increasing the expression of HO-1, these therapies can reduce oxidative damage and promote the clearance of free heme. By upregulating GST and NQO1, Nrf2 activators can enhance the detoxification of ROS and other harmful substances. These combined effects make Nrf2-targeted therapies a comprehensive approach for managing the complex pathology of SCA.[71-73]

Conclusion

The Nrf2 (Nuclear factor erythroid 2-related factor 2) signaling pathway holds significant therapeutic promise for the management of Sickle Cell Anemia (SCA) due to its crucial role in maintaining redox homeostasis and protecting cells from oxidative stress. In SCA, the sickling of red blood cells, hemolysis, and vaso-occlusive crises generate high levels of reactive oxygen species (ROS), which contribute to the disease's severe complications. Nrf2, a key regulator of antioxidant and detoxification responses, offers a novel target for therapeutic interventions aimed at mitigating oxidative damage and improving patient outcomes. Preclinical studies have

demonstrated that Nrf2 activation through pharmacological agents such as bardoxolone methyl and dimethyl fumarate can effectively enhance the cellular antioxidant defenses, reduce oxidative stress, and alleviate symptoms associated with SCA. These compounds activate Nrf2 by modifying Keap1, leading to increased expression of downstream antioxidant genes, including heme oxygenase-1 (HO-1), glutathione S-transferase (GST), and NAD(P)H oxidoreductase 1 (NQO1). The activation of Nrf2 results in the reduction of oxidative damage, improved red blood cell function, and a decrease in the frequency and severity of vaso-occlusive crises in animal models of SCA.

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