Defending against Cellular Damage with Nrf2: A Potential Path for Diabetes Treatment?

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ABSTRACT: The progression of diabetes mellitus — a major disease impacting a significant fraction of the human population — is associated with oxidative damage in the cell caused by free radicals from heightened blood sugar. The protein Nrf2 is a key molecular player in the regulation of the antioxidant response that combats this stress at the cellular level. It achieves this by transcriptionally regulating the expression of genes that control or intersect with signaling pathways that protect the cells. Therefore, Nrf2 regulatory mechanisms have been at the forefront of recent scientific investigations aiming to target diseases stemming from oxidative stress. Animal models show that Nrf2 induction alleviates redox imbalance and alleviates diabetic complications. Conversely, knocking out the Nrf2 mechanism elevates oxidative stress-induced injury to cells. While the evidence so far points to a promising path forward for diabetes treatment, caution is nevertheless warranted in the development of Nrf2-based therapies. This article provides a simple introduction to the cellular machinery responsible for antioxidant functions and reviews the status of current research efforts and the potential for future medical treatments.

KEYWORDS: Biomedical and Health Sciences; Translational Medical Sciences; Genetics and Molecular Biology of Disease; Disease Treatment and Therapies; Diabetes; Oxidative Stress; Antioxidant Response; Nrf2.

Introduction

According to estimates in the National Diabetes Statistics Report¹, 37.3 million Americans, or 11.3% of the US population, were living with diabetes in 2019. In addition, 96 million people age 18 or older were estimated to be prediabetic – 38% of the adult US population. In 2017, the American Diabetes Association estimated the total costs of diagnosed diabetes at $327 billion, and this number is up 26% from the previous estimate in 2012.²-³ Global statistics are no better, with the International Diabetes Federation reporting⁴ 10% of the adult population, or 537 million individuals (20-79 years) living with diabetes in 2021. This number is predicted to rise to 643 million by 2030 and 783 million by 2045. Diabetes has caused at least $966 billion in health expenditures – a 316% jump in the last 15 years. These statistics paint a grim picture which is cause for global concern. The American Diabetes Association highlights its stance by stating that diabetes isn’t a disease – it is an epidemic. Therefore, urgent research efforts are needed to devise solutions for the treatment and management of this disease.

In the US, diabetes is the seventh leading cause of death. Figure 1 shows the percentage of total deaths attributed to diabetes mellitus on a year-by-year basis as sourced from CDC data regarding my home state of Arizona. These numbers show a significant jump in the last 10 years and exceed the national average. The jump in 2020 may be attributed to the comorbidity interplay between COVID-19 and diabetes. Another point of note here is that Arizona is home to a large Hispanic population, and demographically Mexican American adults have almost twice the rate of diagnosed diabetes compared to non-Hispanic white population statistics. This drives home the point that ethnicity, socio-economic status, and access to healthcare are significant factors in worsening the diabetes epidemic.

Despite the formidable challenge, some interesting research avenues have recently opened up to combat the diabetes epidemic. Among them, the relatively new field of Nrf2 research has become a promising area to search for ways to prevent diabetic complications. In contrast to existing medical reviews, this paper provides an easily understandable introduction to the terminology and cellular mechanisms of Nrf2 for a broad audience. An unbiased review of the current status of the research and clinical efforts is presented to the readers for contemplation. The potential of Nrf2 for diabetes treatment is critically evaluated. This includes analyzing the efficacy and side effects of current Nrf2 activation (through genetic and pharmaceutical...
**Discussion**

**What is Diabetes?:**

Diabetes as a medical condition was discussed by Egyptians around 1500 B.C.E, Indian Ayurvedic practitioners around 500 B.C.E, and several others in the following centuries. However, the source of this disease remained unclear until a formal identification of the role of the pancreas was made in 1881 by Joseph von Mering and Oskar Minkowski.\(^5\) The insulin treatment was discovered in 1921, which made it possible for people with diabetes to have longer lifespans.

Diabetes mellitus is a chronic condition, where the body cannot metabolize sugars due to a lack of insulin production by the pancreas or a lack of cellular insulin response. As a result, blood sugar levels rise and can cause serious health complications and death.\(^6\) There are two main types of diabetes mellitus: Type 1—where the pancreas can't make insulin due to the loss of beta cells—and Type 2—where cells develop insulin resistance. In Type 1 patients, administering insulin is necessary every day to survive, whereas Type 2 diabetes can be dealt with or prevented by adhering to a healthy lifestyle.

The depletion of beta cells in Type 1 is caused by an autoimmune disorder, where both genetic and environmental factors are at play.\(^7,8\) The underlying mechanisms are currently a gray area, and more research is needed. However, most reports cite oxidative damage as a significant factor in the progression of diabetes, as the inflammation caused by the cellular damage leads to many complications.\(^9-12\) The evidence from animal models\(^13\) indicates that high glucose levels produce reactive oxygen species which cause oxidative damage, potentially leading to diabetic complications, such as nephropathy (deterioration of kidney function) and cardiomyopathy (heart muscle disease). While this hypothesis was put forward two decades ago,\(^14,15\) recent studies have improved our understanding of oxidative stress in mediating secondary complications.\(^16\) Next, we come to the body’s defense mechanism against oxidation, where the vital component mediating cellular mechanism is a protein called Nrf2.\(^17,18\)

**What is Nrf2, and how does it protect cells?:**

Nrf2 stands for nuclear factor erythroid 2 - related factor 2, and it is a transcription factor that was discovered in 1994.\(^19\) Transcription is the process of making an RNA copy of a gene’s DNA sequence. This copy is called messenger RNA (mRNA), and it carries the gene’s protein information.\(^20\) Among other functions, Nrf2 enables the transcription of genes that encode the antioxidant proteins that protect cells from harmful agents. As such, Nrf2 has been termed the master regulator of the antioxidant response.\(^21\)

Under basal (normal) conditions, Nrf2 is kept in the cytoplasm of the cell, away from the nucleus. This prevents it from affecting its target genes. The Kelch-like-ECH-associated-protein 1 (Keap1) and other proteins form a complex that “locks up” Nrf2, and “marks” it through a process called ubiquitination.\(^22\) Nrf2 is then sent to a proteasome, which degrades it and recycles its components.\(^23,24\) Through this, Nrf2 for,\(^27,28\) Figure 2 schematically shows this process. Ideally, once the proteins have been synthesized, they resolve the source of the oxidative stress. With the problem finally gone, Keap1 returns to its normal shape, allowing it to accommodate Nrf2 once more and bring its levels down to normal.

![Figure 2: Nrf2 promoting transcription of the target gene by binding to MaFG and the ARE (Created with BioRender.com).](image)

**Potential for treatments:**

Type 1 diabetes is treated with insulin while type 2 can be managed with an oral medication that decreases the liver’s production of glucose. However, the premise of new medical therapies relevant to this article is that activation of Nrf2 could enhance the cytoprotective functions, thereby saving the cell from damage under hyperglycemic (high blood sugar) conditions.

As discussed in the section above, Nrf2 regulates the genes that encode proteins needed for defending against the oxidative stress caused, for example, by the production of free radicals during the hyperglycemic state. This begs the question: Can we intervene and control this pathway by activating Nrf2 mechanisms through pharmaceuticals, thus providing a better defense against oxidation induced by hyperglycemia?\(^2\) If we achieve this, it could result in the mitigation of oxidative damage, in turn lowering the risk of diabetic complications. Realizing the potential of this approach, researchers have been targeting the Nrf2 pathway from different angles.

The first approach involves a genetic intervention, through the knockout of the Keap1 gene. Without inhibitory mechanisms, the cell would no longer be able to downregulate Nrf2 activity. At the same time, a large amount of effort is being dedicated to the development of pharmaceuticals to activate the Nrf2 mechanism, with some already being used.\(^29\) Preclinical studies in animal models, mostly based on mice and rats as test systems, have explored a host of synthetic and natural compounds, to explore the possibilities of Nrf2 activation and the protective action it offers. Negi et al.\(^30\) have tabulated a summary of 39 studies, ranging from the investigation of simple elements such as Zinc to compounds such as Sulforaphane, Resveratrol, Curcumin, etc. The results of these studies indicate a reduction of oxidative damage, a lower incidence of diabetic complications, or improved renal and cardiac functioning – in general demonstrating enhanced cellular protection.

Many researchers have focused on the testing of natural products\(^31,32\) in both *in-vivo* and *in-vitro* studies, and again the studies indicate generally positive outcomes. A recent review\(^33\) summarizes many such studies to draw broad conclusions about the role of Nrf2 and the application of natural compounds to activate Nrf2 pathways. In particular, diabetic nephropathy and retinopathy data have been quite convincing
in terms of the advantageous effect of the Nrf2 activation. At the same time, deficiency of Nrf2 shows aggravation of diabetic complications. The evidence, therefore, seems to be strong that Nrf2 activation is the key to the treatment and management of diabetes. In fact, several natural products touting the advantage of Nrf2 activation to consumers are already on the market and are being sold alongside supplements, vitamins, and antioxidants.

Need for caution:
Despite the promise of Nrf2 activation therapy there are several reasons why a cautious approach may be required. A genetic alteration means the cell loses its ability to inhibit Nrf2 through Keap1. This prolongs the activation, and is likely to be detrimental since Nrf2 is an important protein that needs to be tightly regulated for other cellular functions.

Matzinger et al. summarized the results of various studies related to diabetic complications, and in most cases they concluded by saying that there is no direct proof that the activated Nrf2 accounts for all the beneficial effects observed. Similarly, while research indicates that Nrf2 activation lends protection to beta cells in the pancreas, only a few studies show a causal link between the two.

Another issue that needs to be kept in mind is that many treatments have yet to go through clinical trials. In one case, bardoxolone methyl, an Nrf2 activation agent, resulted in an improvement of renal function in diabetic nephropathy patients, but the next stage of the clinical trial had to be terminated due to negative cardiovascular events. The underlying issue often is that such compounds lack specificity in Nrf2 induction and can activate many other off-target proteins causing unintended side effects. There are other clinical trials underway to explore the role of Nrf2 activators in reducing diabetic complications, for example, the sulforaphane in broccoli sprout extract, and the results from these may help to resolve the outstanding questions.

Nrf2 induction treatments have also been tested in relation to a variety of other Nrf2-linked diseases. A major success with Nrf2 activators has been the FDA approval of dimethyl fumarate (DMF) as a treatment for multiple sclerosis. However, it is important to note that because Nrf2 bolsters cellular protection, an overactivity in cancer cells can give them a survival advantage and resistance to chemotherapy. Therefore, the development of a therapeutic strategy for diabetic complications needs to be done carefully with an understanding of beneficial and detrimental effects, and the role of dosage and timing in treatment must also be studied.

Conclusion
Understanding and treating diabetes is vital given its immense impact on our society. It is clear that life expectancy is reduced by diabetic complications arising from oxidative damage in a hyperglycemic state. This article highlights the role of Nrf2 protein as a master regulator of the protective antioxidant effect. Mechanisms by which Nrf2 offers cellular protection are discussed in a simple and easy-to-understand way. The results of previous studies are summarized, which demonstrate that the activation of Nrf2 by synthetic or natural products has a beneficial impact in terms of reducing are several companies already selling Nrf2 activation products in the form of supplements, the author believes that caution is warranted at this stage. Most importantly, the pressing need to develop second-generation Nrf2 activators with higher specificity and reduced off-target toxicity will hopefully drive further inquiry in this field.

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Author

Sameer Sandhu is a high school senior working towards spreading awareness about diabetes and its prevalence in underserved communities. He recently participated in a research project involving the structure of Nrf2 and has since been interested in exploring its implications for diabetes treatments.