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Nrf2 activation as a future target of therapy for chronic diseases

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ABSTRACT

Chronic inflammation integrally related to oxidative stress has been increasingly recognized as a contributing factor in various chronic diseases such as neurodegenerative diseases, pulmonary diseases, metabolic syndrome, and cardiovascular diseases as well as premature aging. Thus, inhibiting this vicious circle has the potential to delay, prevent progression, and treat those diseases. However, adverse effects of current anti-inflammatory drugs and the failure of exogenous antioxidant encourage scientists to develop new therapeutic alternatives.

The nuclear factor E2-related factor 2 (Nrf2) is the transcription factor that is responsible for the expression of antioxidant response element (ARE)-regulated genes and have been described as having many therapeutic effects. In this review, we have discussed the role of oxidative stress in various chronic diseases. Furthermore, we have also explored various novel ways to activate Nrf2 either directly or indirectly, which may have therapeutic potential in attenuating oxidative stress, inflammation and mitochondrial dysfunction that contributes to chronic diseases.

Keywords: Oxidative stress, Mitochondria, Inflammation, Nrf2, Nutrition, Chronic diseases

BACKGROUND

It is widely accepted that chronic diseases including neurodegenerative diseases, obesity, pulmonary diseases, cardiovascular diseases and chronic pain result from a combination of various genetic, lifestyle, and environmental factors (1). However, mounting evidence supports the theory that oxidative stress and chronic inflammation may act as a primary etiologic factor in these diseases (2, 3).

Oxidative stress can be defined as an imbalance between reactive oxygen species (ROS) production and the efficient scavenging of these species by cellular defensive mechanisms, which include both enzymatic scavengers (e.g., superoxide dismutases (SOD), catalase, glutathione peroxidase, glutathione reductases, and peroxiredoxins) and low-molecular-weight reductants (e.g., vitamin E, glutathione, and ascorbate) (4) (Fig.1).

Mitochondria (Mt) use approximately 90% of total oxygen, thus representing the main site of oxygen consumption as well as a primary and continuous source of cellular ROS (5). The rest of intracellular ROS arise from the activity of oxidative enzymes, including the cytochrome P450 system, the cytoplasmatic xanthine oxidase and the membrane enzyme Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase (6). The most important free radicals are oxygen derivatives, particularly superoxide anion (O_2^{-}•), hydroxyl radical (OH^{-}) and hydrogen peroxide (H_2O_2), as well as reactive nitrogen species such as nitric oxide and peroxynitrite.

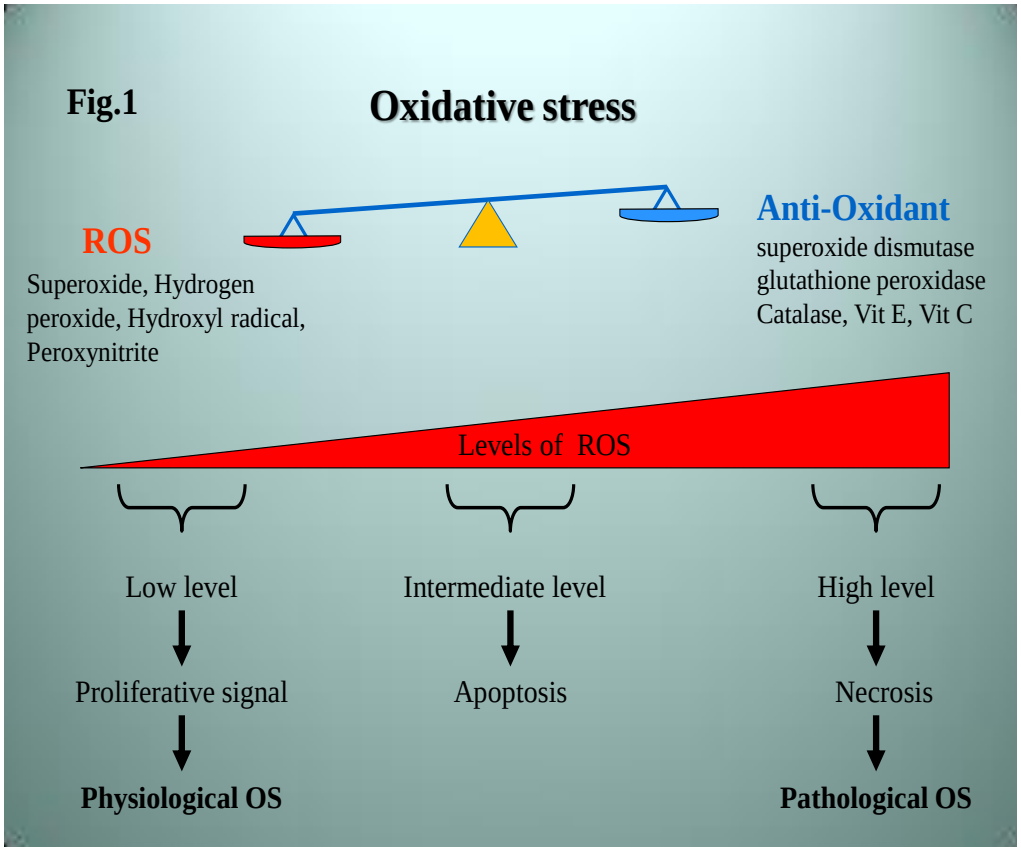

Figure 1. Oxidative stress can be defined as an imbalance between reactive oxygen species (ROS) production and anti-oxidant defense

In normal physiological conditions, a low level of ROS is vital to maintain proper cellular function (7). In contrast, a progressive and irreversible generation of ROS increase to a level where the antioxidant system cannot effectively counterbalance high ROS production. This pathological condition can modify nucleic acids, lipids and proteins, which can have an impact on the pathogenesis of various chronic diseases (8-12).

As a consequence, progressive oxidative stress leads to disruption of mitochondrial structure and function through mitochondrial Deoxyribonucleic Acid (DNA) mutation, Mt (Ca^{2+}) overload and apoptosis (13-18). The role of the mitochondrion in generating and responding to oxidative stress has made mitochondria a promising target for drug delivery and therapeutic intervention.

Chronic inflammatory conditions represent a major disease burden in the Western world, and increasingly, also in developing countries. The importance of inflammatory process for the pathogenesis of human diseases has long been recognized (17-19). Inflammation and oxidative

stress are two sides of the same coin, at least within the context of cells and tissues in vivo (20). Inflammatory cells migrate to the site of inflammation, generating ROS. The resulting oxidative stress recruits more inflammatory cells to their microenvironment. Furthermore, more generation of ROS in turn drives more production and activation of proinflammatory mediators, further enhancing this vicious circle (21, 22). Such ROS/inflammatory cytokine vicious circle may contribute to maintaining and exacerbation of tissue damage (23, 24).

This article will review the networks surrounding oxidative stress related to chronic disease development on which prevention or treatment for early diseases can be managed with possible modulation of the ROS/inflammation vicious circle. We also provide an overview of the promising preventive and therapeutic opportunities of Nrf2 natural and synthetic inducers as disease-modifying molecules. Finally, we will discuss issues that need to be addressed for Nrf2 activators to have their optimal use for human benefit.

PROPOSED MECHANISM OF OXIDATIVE STRESS IN CHRONIC DISEASES

Our proposed hypothesis is that a combination of various genetic, environmental factors as well as inter-dependent mechanisms, including chronic inflammation and oxidative stress, play a significant role in the development and progression of chronic diseases. The potential impact of Nrf2 activation aimed at restoring the redox homeostasis and preventing inflammation is also supported (Fig. 2).

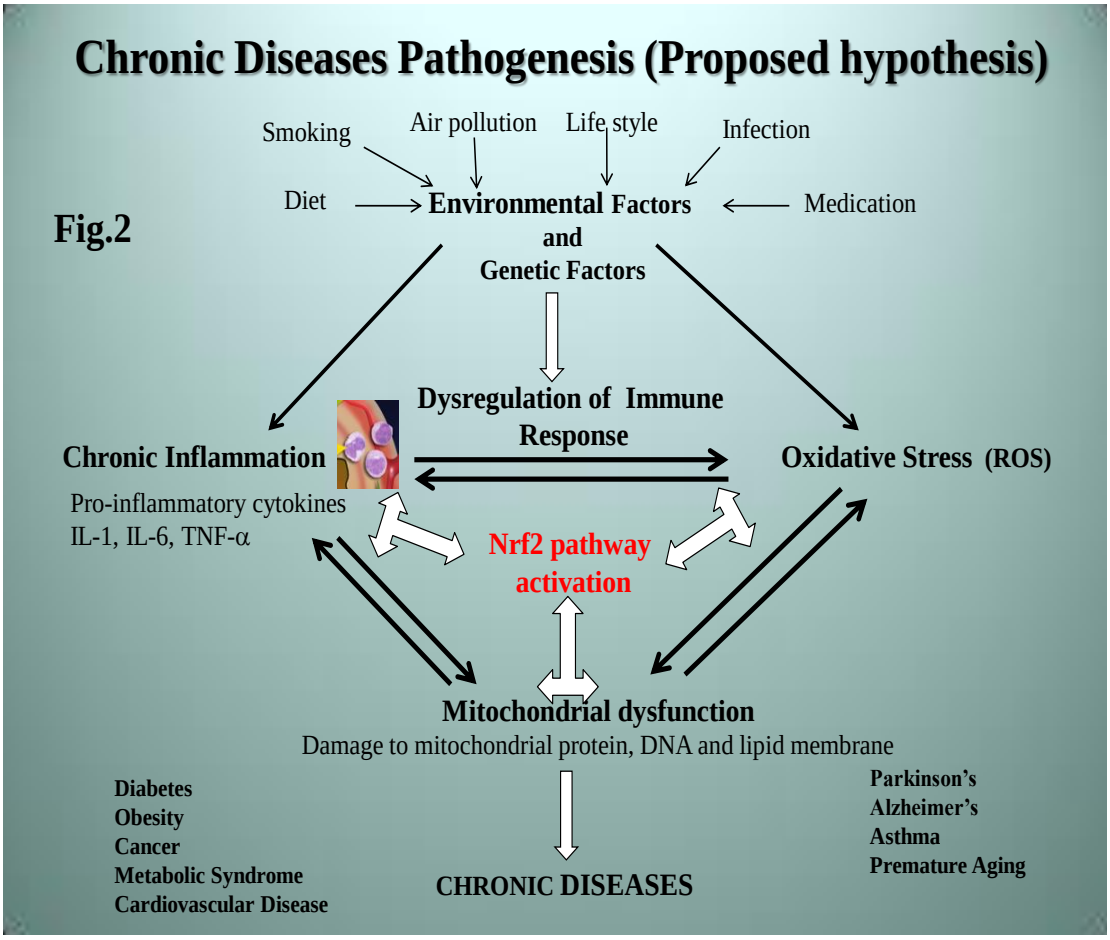

Figure 2. Proposed hypothesis of chronic diseases pathogenesis

In general, genetically susceptible individuals exposed to environmental insults including diet, smoking, air pollution, life style, infection and medication leads to dysfunction of immune response. Immune system disturbance activates inflammatory cells leading to production of pro-inflammatory mediators including TNF-α, IL-6 and IL-1 as well as excessive generation of ROS. Further production of ROS, in turn, recruits more inflammatory cells in a vicious circle. Consequently, with the passage of time, progressive oxidative stress enhanced in chronic inflammation will lead to mitochondrial dysfunction. Oxidative damage modifies a large variety of proteins including mitochondrial proteins and proteins that are involved in signal transduction, which is often irreversible. In addition, oxidative damage to nucleic acids can occur. Particularly mitochondrial DNA is highly susceptible to ROS induced damage because it is located in close proximity to the production site of ROS and mtDNA repair mechanisms are limited. Ultimately, the net result of these processes will lead to a decline in mitochondrial function and concomitant enhanced ROS production (25). Thus, mitochondrial dysfunction may be an important early event in the development of most chronic diseases.

It will be worthwhile to consider how the vicious and synergistic cycle of oxidative stress, chronic inflammation and diminished mitochondrial biogenesis could be modulated to ameliorate chronic diseases. Central to these considerations is the role of the transcription factor Nrf2 and its inhibitor Kelch-like ECH-associated protein1 (Keap1) in regulating the homeostatic response of the organism to many types of stresses, whether they are inflammatory or oxidative. Nrf2 controls the expression of a large number of genes that enable a coordinated protective response to stress. Thus, based on such mechanistic understanding, we can now suggest that Nrf2 is a highly promising candidate for human disease prevention.

TARGETING OXIDATIVE STRESS BY NRF2 ACTIVATION IN CHRONIC DISEASES

In physiological condition, Nrf2 is sequestered by Keap1. Keap1 is a key regulator of the Nrf2 signalling pathway and serves as a molecular switch to turn on and off the Nrf2/Keap1-ARE pathway. When oxidative modification of one of the Keap1 cysteines occurs, Nrf2 escapes from this proteolytic pathway, then translocates to the nucleus, where it dimerizes with a small Maf protein and binds to ARE (26, 27, 28). In pathological condition, degradation of Nrf2 will take place in the cytoplasm once it is released from Keap-1 and never translocate to the nucleus (29, 30).

Two main mechanisms have been proposed for the activation of Nrf2-Keap1 pathway: First, Keap1 contains reactive cysteines following reaction with electrophiles, leading to disruption of the Keap1-Nrf2 interaction and release of Nrf2, which allows Nrf2 entry into the nucleus (31, 32). The second mechanism involves activation of protein kinase signaling pathways resulting in phosphorylation of Nrf2, enhanced release of Nrf2 from Keap1 (33) (Fig. 3).

Activation of Nrf2/ARE pathway should ameliorate a number of diseases driven by inflammatory and oxidative stress processes. We will provide below an updated brief summary on the potential use of Nrf2 to be a target to intervene in a wide variety of preclinical disease models such as neurodegenerative, pulmonary, obesity, metabolic syndrome, cardiovascular and aging.

All the neurodegenerative diseases such as Alzheimer's disease (AD), stroke, multiple sclerosis (MS), Parkinson's disease (PD), and Huntington's disease (HD) have the common features of pathogenesis that include genetic alterations, inflammation, oxidative stress and mitochondrial dysfunction. However, oxidative stress is recognized as a common trigger and is a proposed mechanism for degenerative processes (34-36).

In the context of oxidative stress, hallmarks of oxidative neuronal injury have been found in a range of disorders, including AD, ALS and PD (34, 35). In addition, it has been observed that oxidized glutathione is significantly higher in blood cells from PD patients as compared to controls and concentrations of antioxidant catalase activity are decreased (36). Despite these established links between neurodegenerative disease to oxidative stress, trials of antioxidants have had limited success. As such, scientists are turning towards the mechanisms by enhancing endogenous antioxidant defenses.

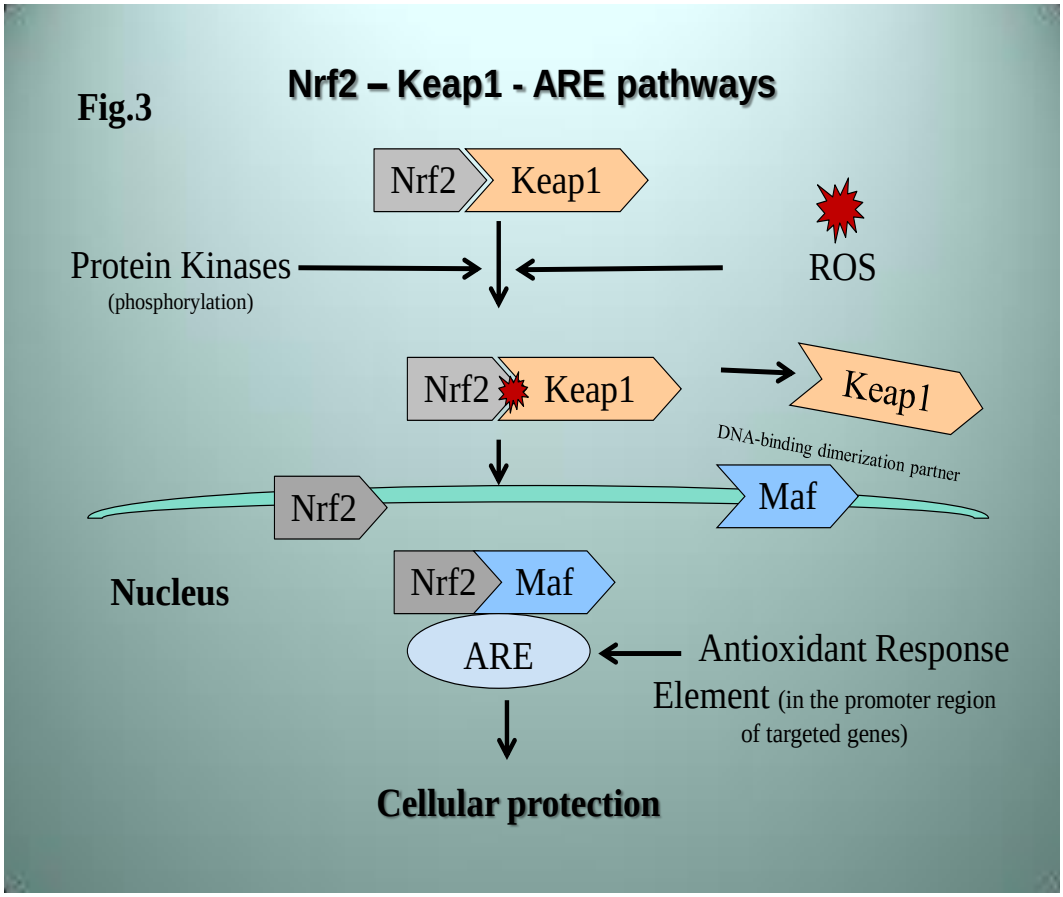

Figure 3. Nrf2 (nuclear factor erythroid 2 related factor) is a redox sensitive, basic leucine zipper transcription factor, which is normally attached to Keap1 in the cytoplasm. With low levels of oxidation or phosphorylation, it dissociates from Keap1 (inhibitor of Nrf2) and enters the nucleus to attach to ARE in the promoter region of target genes to induce endogenous antioxidant activities.

Recent experimental PD model studies have indicated that activation of endogenous antioxidant gene expression, in particular those under the control of the Nrf2 pathway, has more neuroprotective effects as compared to conventional antioxidant therapy (37, 38, 39).

Oxidative stress and increased airway inflammation has been postulated to play an important role in the pathogenesis of pulmonary diseases including asthma and chronic obstructive pulmonary diseases (COPD). Genetic inactivation of Nrf2 pathway exacerbates airway inflammation in the airways of Nrf2 (-/-) mice (40). Rangasamy has demonstrated that blockage of the Nrf2 gene leads to severe allergen-driven airway inflammation and hyper-responsiveness in a mice model of asthma. Enhanced asthmatic response as a result of ovalbumin sensitization and challenge in Nrf2-disrupted mice was associated with more infiltration of eosinophils, IL-4 and IL-13 into the lungs than seen in wild-type mice (41).

In a preclinical model of COPD, various data have identified up-regulation of Nrf2 inducible genes as a protective molecule against oxidative stress in the lung only in Nrf2 (+/+) mice. In contrast, this protective role is missing in Nrf2 (-/-) mice. Hence, Nrf2 targeting might provide clinical value by suppressing both airway inflammation and oxidative stress and attenuating the progression of diseases in experimental models of asthma and COPD (41, 42).

Oxidative stress is a primary contributor to the development and progression of cardiovascular diseases. Strategies acting directly on ROS, by either reducing the production or increasing the elimination, can be an important therapeutic target for the treatment of cardiovascular diseases. Activation of the Nrf2 pathway is also useful for maintaining homeostasis in the cardiovascular system. It has been observed that cigarette smoking triggers cardiac dysfunction in the right ventricle that is worse in Nrf2 (-/-) mice than in Nrf2 (+/+) mice, thus it seems that Nrf2 reduces cardiac damage from smoking (43). Accordingly, the metabolic induction of Nrf2 pathway promises to be a viable therapy for attenuating oxidative stress-mediated damage in cardiovascular diseases.

Genetic and pharmacological activation of Nrf2 pathway induce a number of genes involved in lipid metabolism and are beneficial in preliminary animal studies of obesity and diabetes. Nrf2 activation in preclinical studies contributes to the control of lipid metabolism under both normal and high-fat diet conditions (44, 45, 46).

It has been suggested that oxidative stress may be the mechanistic link between obesity and related diseases (47). Obesity is a major risk factor for both type 2 diabetes and metabolic syndrome. This syndrome is a complex disorder combining obesity, dyslipidemia, hypertension, and insulin resistance (48). The reduction in obesity in the group treated with Nrf2 activator is accompanied by increased energy expenditure, but these effects are lost in Nrf2 deficient mice (49). More recently, the activation of Nrf2 pathway has become a popular means for potential intervention for metabolic syndrome treatment (50).

One of the putative basic mechanisms of aging is mitochondrial deterioration mediated by ROS. Thus, there is great interest in approaches to protect mitochondria from ROS-mediated damage in pre-aging condition (51).

The free radical theory of aging proposes that several age-related changes in immune cell functions, which depend on the redox state of these cells, could be good markers of health, biological age and longevity (52, 53). Thus, enhancing endogenous antioxidant capacity and cellular stress response by Nrf2 activation could reverse the vicious circle of oxidative stress and inflammation in vascular aging. A recent study has suggested that metformin promoted health span extension by activating antioxidant defense longevity pathways. It is possible that

metformin may be a plausible pharmacological intervention to promote healthy human aging through activation of Nrf2 signalling pathway (54).

In recent years interest from academic institutes and pharmaceutical companies is rising to test the efficacy and safety of Nrf2 activators, including Bardoxolone methyl and Fumaric acid, in clinical trials in chronic diseases including diabetic nephropathy and multiple sclerosis.

Bardoxolone methyl was entered into phase III clinical trials in patients with type II diabetes and chronic kidney disease (55). Oral diethyl fume rate is the active compound of BG-12 that was recently licensed for the treatment of relapsing-remitting multiple sclerosis. It dramatically reduced relapse rates and it also reduced lesions detected by MRI as well as a range of measures of disability progression (56). The mode of action is not exactly clear, but an activation of the transcription factor Nrf2 was suggested.

While Nrf2 may not be clinically validated, these two clinical trials have further stimulated research efforts, and the theory continues to highlight this pathway as an exciting future source of therapies for a range of diseases. This approach now needs to be extended to the prevention of inflammatory and oxidative stress related diseases. Finally, it will be worthwhile to accelerate successful clinical translation of the Nrf2 therapeutics pathway.

THERAPEUTIC APPROACHES TARGETING OXIDATIVE DAMAGE IN CHRONIC DISEASES:

Mitochondria produce the energy needed for normal cellular function and metabolic homeostasis by mitochondrial oxidative phosphorylation (OXPHOS) and serve as biosensors for oxidative stress (57-59). It has been well indicated that mitochondria are the major source of intracellular ROS and are most vulnerable to oxidative damage. Thus, it would be ideal to deliver the antioxidant therapy to mitochondria. There have been many strategies exploited to target therapeutic agents to mitochondria. However, mitochondrial-targeted antioxidants research is still in its infancy and it will take a considerable amount of time to have more promising data.

Adverse effects of current anti-inflammatory drugs and the failure of exogenous antioxidants to prevent or stop progression of chronic diseases have encouraged academic and pharmaceutical investigators to develop a new therapeutic approach (Fig. 4.). It is important to emphasize the need for alternative strategies to therapeutically counteract the oxidative stress-mediated diseases. Therapeutic interventions to enhance endogenous antioxidants capacity by Nrf2 factor are a valuable alternative mechanism to the use of exogenous antioxidants to reverse the vicious circle of oxidative stress and inflammation. The Nrf2/ARE pathway can be pharmacologically activated by molecules of natural derivation (nutraceuticals) and by chemically synthesized drug products.

The mechanism of nutraceuticals to counteract oxidative stress and promote cell survival signals have been demonstrated in many *in vitro* and *in vivo* models of various chronic diseases (60-62). Given that chronic inflammation and oxidative stress is a principal cause of chronic diseases, effective natural antioxidants and anti-inflammatory agents could provide novel and safe therapeutic options for these devastating disorders.

Although evidence shows that antioxidant treatment results in cytoprotection, the potential clinical benefit deriving from both nutritional and supplemental antioxidants is still under wide debate (63). However, recent research has shown that dietary polyphenols, naturally occurring

phytochemicals like curcumin, caffeic acid phenyl ester, sulforaphane, triterpenoids and tocopherol, have been used to activate the Nrf2/ARE pathway and are widely used to ameliorate excessive oxidative stress mainly through enhancing endogenous antioxidant.

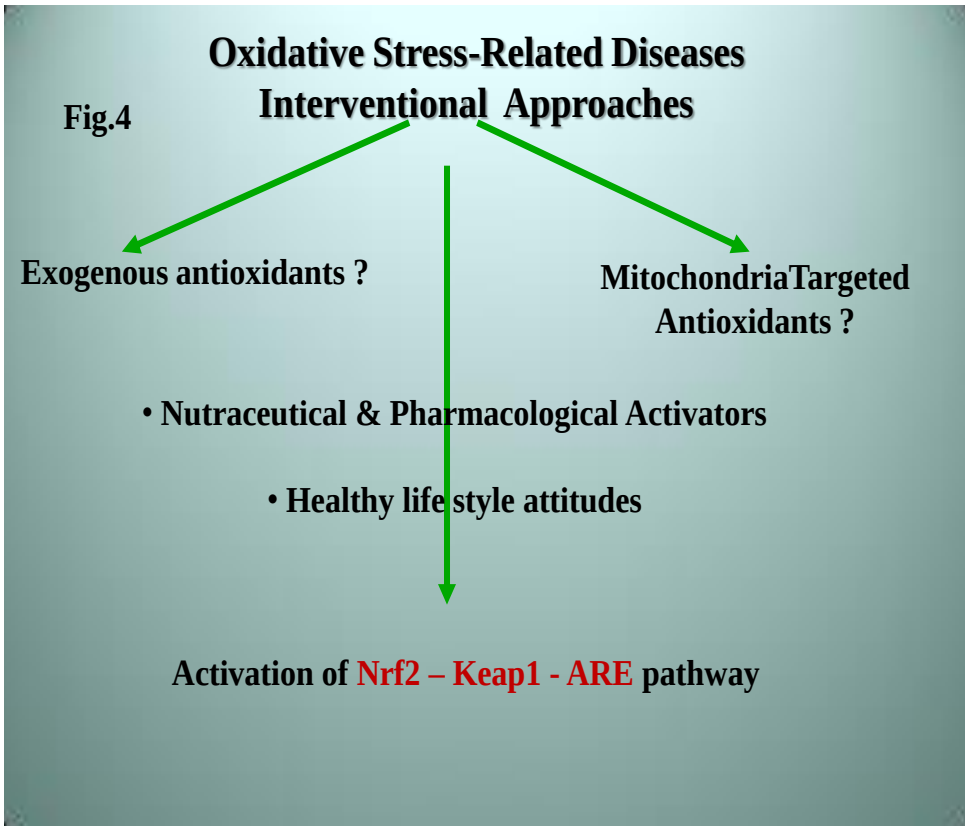

Figure 4. Therapeutic approach of chronic diseases: Role of Nrf2

Consumption of fruits, vegetables and other foods and beverages including apples, berries, broccoli, Brussels sprouts, cauliflower, onions, herbs, cocoa, tea and red wine provides an important source of Nrf2 and has been found as having many therapeutic effects via induction of key enzymes involved in cellular antioxidant/anti-inflammatory network. Many of these same enzymes also appear to play a key role in maintenance and redox recycling of essential Vit A, C, and E (64-66). Thus, increased consumption of cruciferous vegetables has been associated with a decreased risk of several age related diseases including cardiovascular, degenerative diseases and cancer.

Systematic reviews provide strong evidence that life style interventions are successful in preventing and decreasing the risk for cardiovascular diseases, obesity, diabetes mellitus and metabolic syndrome (27). Lifestyle interventions that include moderate caloric restriction along with aerobic and resistance exercise have shown improvements in metabolic outcomes, strength, and physical function in older adults who are obese.

Recent observations by Gounder SS et al observed that old mice were highly susceptible to oxidative stress following high endurance exercise stress, but demonstrated increased adaptive redox homeostasis after moderate exercise training for 6 weeks suggesting that enhancing Nrf2 function and endogenous cytoprotective mechanisms by moderate exercise training may protect the myocardium from oxidative stress complications (68).

It has been observed that sedentary older humans exhibit Nrf2-Keap1 dysfunction, but an active life style increases Nrf2 function and thereby maintains redox homeostasis in skeletal muscle of older humans. Safdar A et al, proposed that the metabolic induction of Nrf2-Keap1 redox signalling promises to be a viable therapy for attenuating oxidative stress-mediated damage in skeletal muscle associated with physical inactivity (69).

We believe that a physically active lifestyle promotes adaptations throughout the aging process via activation of Nrf2/Keap1 pathway, enhancing antioxidant defence that allow the cell to maintain energy homeostasis despite the accumulation of abnormal mitochondrial protein, DNA and lipid peroxidation.

CONCLUSION AND FUTURE PERSPECTIVE

We are urgently needed to alter the patterns of medical practice to a more preventive orientation because increasing costs of treating chronic diseases impose increasingly unsustainable economic burdens on society.

The development of a specific Nrf2 activator would bring novel approach for the prevention/treatment of a wide variety of chronic human diseases and may be part of the new era for the biomedical science and should be used as early as possible in the pathogenesis of any diseases, when a homeostatic agent is appropriate.

Although Nrf2 activation represents promising therapeutic targets, their role in the regulation of mammalian lifespan remain an open question. Animal models and clinical studies will be needed to identify the efficacy and safety of Nrf2 activators in the prevention and/or treatment of chronic diseases.

We suggest that pharmacological and natural Nrf2 inducers may provide a novel therapeutic strategy for restoring cellular redox homeostasis and a promising therapeutic strategy in aging and various chronic diseases.

Finally, we suggest that nutrition and healthy lifestyle educational interventions can be a successful and effective complementary approach to overcome defective antioxidant response and inflammation in chronic diseases.

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