NRF-2: A NOVEL REGULATOR OF THE IMMUNE SYSTEM

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Abstract

Nuclear factor-erythroid 2-related factor-2 (Nrf-2) is a transcription factor that plays a central role in cellular defense against oxidative and electrophilic insults by induction of antioxidative and phase-2 detoxifying enzymes. Inhibition of Nrf-2 signaling renders the organism susceptible not only to oxidative and electrophilic stresses but also results in dysregulated inflammatory responses. Activation of Nrf-2 pathway has been shown to be helpful in protection against inflammatory and auto-immune disorders. Hence, induction of Nrf-2 using redox active agents could be considered as effective means for the prevention and treatment of inflammation associated diseases.

Introduction

The well-known hallmarks of inflammation i.e. redness, swelling, heat, pain were described over 2000 years ago by Celsus and are characteristic of any kind of infection or injury. During an immune response, these symptoms of acute inflammation dissipate swiftly after the infection is cleared and the damaged tissue is repaired. However, during chronic inflammation the immune system goes into overdrive because the stimulus that triggers the inflammation persists for long time and contributes substantially to chronic diseases. While acute inflammation is an important element of the organism’s defense repertoire, persistent inflammation can contribute substantially to the pathogenesis of several age-related chronic diseases such as metabolic diseases, type 2 diabetes, cardiovascular diseases, and neurodegenerative diseases. Suppression of this superfluous and chronic activation of immune cells by using immunosuppressive agents is desirable to avoid occurrence of the aforementioned inflammation associated disorders. In addition to this, successful organ transplantation also requires the use of immunosuppressive drugs to prevent the host's immune system from rejecting the transplanted organ. Immunosuppressive agents involve many classes of drugs such as inhibitors of calcium signaling and translation (Cyclosporin A, tacrolimus), inhibitors of nucleotide synthesis (azathioprine, mycophenolate mofetil), inhibitors of growth factor signal transduction (sirolimus) and also antibody therapies that eliminate specific groups of cells. Each of these drugs induces a distinct set of side effects on normal tissues which limits their long term usage and hence there is a need for identification of novel targets for regulation of chronic inflammation. Over the last decade our lab has been working towards the identification of novel targets to manage uncontrolled inflammatory responses. We have now shown that redox sensitive immuno-regulatory transcription factors can be used as druggable targets for the development of new anti-inflammatory drugs.
Cellular redox and Nrf-2 as regulators of immune responses

Cellular redox status is the ratio of the inter-convertible oxidized and reduced form of intracellular redox couples (GSSG/GSH) that work together to maintain the redox environment which is important for a variety of cellular functions such as proliferation, apoptosis and intracellular signaling. Alteration in reactive oxygen species (ROS) levels and redox status leads to aberrant immune functions. Nuclear factor erythroid 2-related factor 2 (Nrf-2) is a redox-sensitive, basic-leucine zipper transcription factor [1]. It is expressed in a variety of cell types where it contributes to maintenance of redox homeostasis by regulating key cytoprotective/antioxidant genes, including glutathione (GSH), heme oxygenase-1 (HO-1), NAD(P)H:quinone oxidoreductase 1, and superoxide dismutases. In addition, Nrf-2 is also reported to regulate innate immune response that dramatically improves survival during experimental sepsis by protecting against dysregulated inflammation [2]. Under resting conditions, Nrf-2 is sequestered in the cytoplasm by the cytoskeleton-associated protein, Kelch-like ECH-associated protein 1 (Keap1) which functions as a negative regulator of Nrf-2 by promoting the ubiquitination and proteasomal degradation of Nrf-2 [3]. When liberated from its repressor Keap1, Nrf-2 translocates into the nucleus and binds to its consensus sequence in the promoter region of many genes whose products are involved in cellular defence against toxins, oxidative stress and electrophiles. A widely accepted model for nuclear translocation and activation of Nrf-2 involves alteration of the Keap1 structure by oxidation or covalent modification of critical cysteines present in Keap1. We have shown that compounds which do not show cellular toxicity and yet modify Keap1 by inducing redox imbalance may prove to be important candidates for the development of novel anti-inflammatory drugs.

Cross-talk between Nrf-2 and NF-κB

NF-κB is a redox sensitive transcription factor and central regulator of many genes involved in immune response including cytokines, chemokines, cytokine/chemokine receptors, adhesion molecules, survival genes, cell cycle regulators, acute phase proteins, and inducible effector enzymes. Since Nrf-2 is an important transcription factor responsible for maintaining cellular antioxidant capacity, it has been shown that Nrf-2 may inactivate or counteract NF-κB signaling. Pro-inflammatory genes known to be regulated by NF-κB were highly expressed upon lipopolysaccharide stimulation in Nrf-2 knockout mice when compared with those in wild-type mice. Activity of IκB kinase (IKK) and IκBα degradation needed for NF-κB activation were more pronounced in Nrf-2−/− mouse embryo fibroblasts (MEFs) stimulated with LPS or TNF-α [2]. Hence, agents that activate Nrf-2 by directly modifying cysteine thiols of Keap1 may lead to suppression of NF-κB and in addition these agents may also target the critical cysteine residues of p50 or IKK, thereby blocking NF-κB pathway.

Redox active agents and Nrf-2 activators as putative anti-inflammatory drugs

Based on the studies carried out by us and several other investigators, we hypothesized that redox active agents may exhibit potent anti-inflammatory activity by perturbing cellular redox and by activating Nrf-2 pathway. The studies carried out in our laboratory provide convincing evidence for redox modulation as a novel strategy for regulating immune responses and for
development of new anti-inflammatory drugs. Further, we have also shown that phytochemicals can be used to modulate cellular redox balance which would find significant application towards management of immune responses for therapeutic benefits. We have used both synthetic and plant derived molecules like naphthoquinones (plumbagin, menadione, 1,4-naphthoquinone), ursolic acid, schisandrin B etc and studied their anti-inflammatory effects [4-10]. The detailed molecular mechanisms that may contribute to their immuno-modulatory activity were also elucidated. We have for the first time demonstrated that plumbagin, (5-hydroxy-2-methyl-1,4-naphthoquinone), acts as a pro-oxidant in normal lymphocytes and suppresses mitogen induced T-cell activation, proliferation, cell cycle progression and effector functions in vitro and in vivo.

The potent anti-inflammatory effects of plumbagin were attributed to its ability to suppress redox sensitive immuno-regulatory transcription factor NF-κB in lymphocytes and macrophages. It was further revealed that depletion of intracellular glutathione levels in lymphocytes by plumbagin is responsible for the observed oxidative stress. Our results for the first time demonstrated that plumbagin indeed interacts with and forms an adduct with intracellular free thiols. It was also established that plumbagin could modulate thiol groups on critical cysteine residues of proteins by inducing glutathionylation. Plumbagin inhibited nuclear translocation and activation of NF-κB through induction of p65 glutathionylation.

Further, the in vivo anti-inflammatory potential of plumbagin was also verified using mouse models of graft-versus-host-disease, allograft transplant and endotoxin induced septic shock. Our studies highlight the potential application of plumbagin as an anti-inflammatory agent which may be used in the treatment of inflammatory disorders. Further, we also demonstrated that glutathionylation can be an important mechanism by which inflammatory pathways and signalling mediators are regulated and provide new insights into novel anti-inflammatory strategies. In addition to plumbagin, menadione, ursolic acid and schisandrin B were also shown to act as a pro-oxidant in normal lymphocytes and suppresses mitogen induced T-cell activation, proliferation and effector functions in vitro and in vivo.

All the above mentioned agents have been shown to alter redox balance leading to activation of Nrf-2 pathway and upregulation of Nrf-2 dependent genes. In our studies, we have reported that the potent anti-inflammatory effects of these Nrf-2 activator molecules were also due to their ability to suppress redox sensitive immuno-regulatory transcription factor NF-κB in lymphocytes.

In addition to this, we have demonstrated the ability of redox active molecules to ameliorate radiation induced damage to normal cells and tissues. We have shown that, contrary to conventional wisdom of antioxidants acting as radioprotectors, even pro-oxidants which induce mild oxidative stress, could offer radioprotection to normal cells [11]. The ability of these molecules to upregulate intra-cellular anti-oxidant defences and induce multiple pro-survival signalling pathways like ERK and Nrf-2 was shown to be responsible for the observed radioprotection.
Conclusions and Future Direction

Our studies highlight a potential application of redox active molecules as anti-inflammatory agents which may find application in the treatment of inflammatory disorders. Since NF-κB is a central regulator of pro-inflammatory signaling pathways, suppression of NF-κB-mediated transcriptional activity by Nrf-2 is a promising strategy to control dysregulated immune responses.

Our future challenges include development of novel immunosuppressant strategies whereby a wider choice of immunosuppressive agents will probably help physicians to minimize the burden of drug induced toxicity (side effects) in patients. The use of redox active agents derived from natural sources which have a favorable toxicity profile unlike corticosteroids and cytotoxic agents (methotrexate, azathioprine, cyclosporine) will be particularly helpful in treatment of chronic inflammatory conditions.

Scheme: Anti-inflammatory effects of redox active agents are mediated via activation of Nrf-2 and suppression of NF-κB
References