NRF2/NF-κB CROSS TALK: A PUTATIVE TARGET FOR DEVELOPING NOVEL ANTI-INFLAMMATORY AGENTS

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ABSTRACT
Persistent and prolonged inflammation is the root of multiple immune disorders. Though multiple treatment modalities are available based on diverse strategies, still a dire need exists to develop novel anti-inflammatory agents with least side effects. Among the potent strategies employed, activation of Nrf2 and inhibition of NF-κB pathway independently have yielded promising results of immunosuppression. The present article highlights the potential of targeting the cross talk between these two redox sensitive immuno-regulatory transcription factors to develop novel repository of anti-inflammatory agents. Developing an agent that can activate Nrf2 pathway to confer anti-inflammatory/cytoprotective effects and simultaneously inhibit NF-κB pathway to curb inflammation would be superlative over other available modalities.

KEYWORDS: KEAP-1, IKKβ, chronic inflammation, prooxidants.

INTRODUCTION
Inflammation is a complex localized protective reaction initiated by host cells/tissues of the body to allergic or chemical stimulation, injury and infections. Outcome of inflammation is mediated by secretion of chemical mediators from cells like mast cells, platelets, neutrophils and monocytes/macrophages. These mediators are termed as pro-inflammatory factors that determine the severity of inflammation.[1] Importunate and prolonged chronic inflammation is the underlying cause of multiple oxidative stress associated diseases such as rheumatoid arthritis, atherosclerosis, heart disease, Alzheimer, asthma, cancer, congestive heart failure (CHF), multiple sclerosis (MS), diabetes, infections (bacteria, fungi, parasites), gout, IBD-inflammatory bowel disease. Lymphocytes (T cells, B cells), macrophages and neutrophils are the major immune cells that are involved in the manifestation of inflammation.[2,3]
A plethora of effective anti-inflammatory agents are approved for use, including non-steroidal anti-inflammatories, with many more drugs under investigation. In particular, the new era of anti-inflammatory agents includes anti-cytokine therapy, kinase inhibitors, statins, histone deacetylase inhibitors, PPAR agonists and small RNAs.\cite{4} Despite the remarkable advances of the last half-century, current immunosuppressive drugs are not ideal approach to support long term acceptance of solid organ transplant and survival. Each agent is associated with its own set of toxicities in addition to the shared adverse consequences of long-term immunosuppression due to reduction in host defense against infections. Few of the therapeutic modalities have even resulted in progressive multifocal leukoencephalopathy.\cite{5} Further, blocking cytokines may reduce inflammation but also renders the host susceptible to infection and maybe even cancer. Though combinatorial treatment modalities are being explored but it seems unlikely that additional permutations of currently available therapies will be able to alter this dynamic. Thus, a dire need exists to identify a new target that can yield a class of compounds with lesser side effects and more survival. The present article highlights the potential of Nrf2/NF-κB cross talk as prime target to develop new anti-inflammatory agents.

**Nrf2 PATHWAY**

Nuclear factor [erythroid-derived 2]-like 2 (Nrf2) is a redox sensitive immunoregulatory transcription factor. It was first identified as NF-E2-like basic leucine zipper transcriptional activator that binds to the tandem E2/AP1 repeat of the β-globin locus control regions. It was later characterized as Cap’n’Collar (CNC) protein involved in the control of development of *Drosophila* head segment.\cite{6} Nrf2 is sequestered in the cytoplasm by its repressor KEAP-1 which strictly regulates Nrf2 stabilization and maintains the levels inside the cell. A characteristic distinguishing feature of KEAP-1 protein is that it contains numerous cysteine (cys) residues that encrypt its potential to act as a redox sensor.\cite{7} Exposure of cells to low levels of oxidative stress, electrophiles or chemopreventive agents induces dissociation from KEAP-1 and translocation to the nucleus. In nucleus Nrf2 forms a heterodimer with its co-transcription factor Maf and binds to the antioxidant response element (ARE, 5’-RTGACnnnGCR-3’) sequence to induce transcription of several different types of genes. The Nrf2 downstream genes includes intracellular cytoprotective proteins like glutamate cysteine ligase (GCL), glutathione peroxidase (GPx), thioredoxin (Trx), thioredoxin reductase (TrxR), (NQO1), UDP-glucuronosyl transferase (UGT), peroxiredoxin (Prx), hemeoxygenase-1 (HO-1) and NAD(P)H quinone oxidoreductase-1.\cite{8,9}
Nrf2 knockout mice showed enhanced bronchial inflammation, prolonged inflammation during cutaneous wound healing, and high susceptibility for lupus like autoimmune syndrome, enhanced lymphocyte proliferation and impaired redox status. Further, Nrf2 deficient mice showed higher mortality to septic shock caused by lipopolysaccharide or cecal ligation or puncture.\[^{10,11}\] Nrf2 dependent protein, HO-1 and its degradation product carbon monoxide, have been reported to show anti-inflammatory effects by inhibiting pro-inflammatory cytokine production, leukocyte migration, adhesion and suppressed LPS induced production of tumour necrosis factor-a (TNF-a) and nitric oxide (NO) in murine macrophages.\[^{12}\] Thus, Nrf2 activation is a putative target for developing novel anti-inflammatory agents. But, apart from Nrf2 there is another pivotal redox sensitive immunoregulatory transcription factor i.e. NF-κB which is a central regulator in eliciting the inflammatory responses.

**NF-KB PATHWAY**

The heterodimeric protein NF-κB is a ubiquituous redox-regulated transcription factor that remains sequestered in the cytoplasm as an inactive complex with its inhibitory counterpart IkBα. NF-κB is sequestered in cytoplasm by IkBα which contains ankyrin repeats that bind at RHD domain of NF-κB protein masking the nuclear localisation signal and DNA binding motif.\[^{13,14}\] NF-κB can be activated by an array of stimuli like oxidative stress, IR, microbial injection, TNF alpha, IL-1, MHC-peptide-TCR interaction or CD3/CD28 stimulation. It is generally accepted that degradation of IkBα is a pivotal step in NF-κB activation several recent studies have reported that NF-κB may be activated independently of IkBα degradation and phosphorylation of its active subunit p65/RelA. IkBα kinase (IKK) complex further phosphorylates ser residue on IkBα at N terminal region leading to its ubiquitination mediated degradation by 26S proteosome machinery. NF-κB translocates to the nucleus where further post translational changes govern its activity. NF-κB/Rel target genes include cytokines, chemokines, cytokine/chemokine receptors, adhesion molecules, survival genes, cell cycle regulators, acute phase proteins and inducible effector enzymes.\[^{15}\] Activated NF-κB often facilitates transcription of numerous genes, including iNOS, COX-2, interleukin-6 (IL-6), IL-1β, tumor necrosis factor-R, 5-lipoxygenase, hypoxia inducible factor-1R, and vascular endothelial growth factor, resulting in inflammation and tumorigenesis. Blocking of NF-κB activation, thereby inhibiting T cell responses, is used as an important strategy for curbing inflammation using small molecules by several investigators.\[^{16}\]
Cross Talk Between Nrf2 And NF-κB Pathway

An ideal anti-inflammatory agent should be able to curb the inflammatory responses (can be achieved by inhibiting pro-inflammatory transcription factor NF-κB and activating anti-inflammatory transcription factor Nrf2) and should be associated with least side effects (can be achieved by cytoprotective action of Nrf2). Thus, investigating novel agents that can target the cross talk between these two transcription factors may prove as an amenable strategy to curb inflammation with least side effects. Interestingly, activation of Nrf2 has been shown to suppress activation of NF-κB thereby inhibiting inflammatory reactions.

Multiple studies have highlighted the cross talk between Nrf2 and NF-κB as prime target for development of novel anti-inflammatory agents. Kim et al showed the ability of KEAP-1 to suppress NF-κB pathway by inhibiting phosphorylation and inducing degradation of IKKβ. Upon Nrf2 activation, KEAP-1 binds to IKKβ and functions as an adapter protein for CUL-3 based E3 ligase for its degradation, which is responsible for suppression of NF-κB pathway and modulation of immune responses. Several anti-inflammatory phytochemicals suppress NF-κB signaling and activate the Nrf2 pathway. Redox modifiers like prooxidants are putative repositories having the potential of performing dual role of activating Nrf2 and inhibiting NF-κB. Gambhir et al showed that 1, 4 naphthoquinone, a prooxidant, activated Nrf2 and induced KEAP-1 mediated inhibition of NF-κB in murine lymphocytes to suppress mitogen induced inflammatory responses. Further, Nrf2-deficient mice, subjected to a moderately severe head injury, show a greater cerebral NF-κB activation compared with their wild-type Nrf2 counterparts whereas Nrf2 over expression suppressed NF-κB - DNA binding activity. Cinnamaldehyde was shown to activate Nrf2 and inhibit the degradation of IkBα leading to suppression of NF-κB pathway. Curcumin, a polyphenol, was shown to activate Nrf2 which further mediated the modulation of expression and transactivation of NF-Kb. Thus, these studies clearly demonstrate the potential of the cross talk between these two transcription factors as prime target for therapeutic interventions.

CONCLUSION

Perturbation in cellular redox status by redox modifiers to achieve the activation of redox sensitive transcription factors for cytoprotection is among the successful strategies for developing novel drugs. Depending on the extent of modulation in cellular redox levels, regulation of redox sensitive transcription factors can be achieved. Indispensable role of Nrf2 and NF-κB in inflammatory response has been well documented in literature and well
exploited for developing anti-inflammatory agents. However, the investigated agents are associated with side effects due to prolonged immunosuppression and infections. Thus, an agent able to curb inflammation and also confer cytoprotection should prove superlative over other treatment modalities. Interestingly, cross talk between Nrf2 and NF-κB pathway paves the way for investigating such agents. Prooxidant repositories have been shown to activate Nrf2 and subsequently KEAP-1 mediated inhibition of NF-κB pathway to diminish inflammation. However, further studies are warranted in investigating the potent target points in the cross talk between Nrf2 and NF-κB to provide ample opportunities for therapeutic interventions.

**Conflict of Interest:** There is no actual or potential conflict of interest.

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