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Perturbation of Cellular Redox Status: Role of Nrf2, a Master Regulator of Cellular Redox

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Abstract

Regulation of cellular redox homeostasis determines the fate of the cell. Perturbation in redox status is known to elicit multiple cellular pathways. Role of oxidative stress modulation in channelizing the cell towards apoptosis or rescuing the cell by activating pro-survival pathways, depends on the levels of generated oxidative stress. High levels of generated oxidative stress induce cell death pathways whereas mild and low levels are known to elicit the cell survival pathways. Generation of ROS for a short duration of time inducing Redox ticking also triggers the pro-survival pathways inside the cell. Nrf2 is the redox sensitive prosurvival transcription factor which acts as master regulator of redox equilibrium. Nrf2 and its dependent genes including HO-1, GCLC, NQO1 etc. are involved in maintaining the cellular redox homeostasis. Role of Nrf2 as dual edges sword has been highlighted in past decade. The cross talk between the Nrf2 and NF- κ B is at the focal point of building the redox response network. The present chapter is aimed at providing the insight on the role of Nrf2 and NF- κ B as redox sensitive transcription factors in regulating cellular redox status. Further, the chapter brings in light the therapeutic potential of targeting Nrf2 under multiple clinical settings.

Keywords: redox homeostasis, Nrf2, NF- κ B, therapeutic potential, cancer, neurodegeneration

1. Introduction

An equilibrium between the oxidants, reactive oxygen species and antioxidants attained by cell is defined as redox status of the cell, In case of any kinds of diseases or pathological conditions which disrupt this equilibrium thus creating an oxidized state, termed as oxidative stress [1]. This redox system essentially works in maintaining cellular homeostasis and cell survival. Reactive oxygen species (ROS) consist of reactive species like superoxide (O_2^-) and hydroxyl radical (HO), along with nonradical species such as hydrogen peroxide (H_2O_2). Reactive nitrogen species (RNS) contains nitrogen containing reactive species including nitric oxide (NO) and peroxynitrite ($ONOO^-$). Agents that contribute to formation of these ROS/RNS may be exogenous sources like Chemicals (e.g., PCB), irradiation (i.e., UV irradiation, x-ray, gamma-ray) or atmospheric pollutants or they may be endogenous the mitochondria, where O_2^- is generated by electron leakage from complex I and III of the electron-transport

chain, membrane-associated NAD(P)H oxidase, cytochrome c oxidase, and xanthine oxidase. In case of any oxidative stress experienced by the cell various enzymatic and non-enzymatic antioxidant systems present in the cell are ready to combat. A major class of enzymatic antioxidant systems include multiple isoforms of SOD, (SOD1, SOD2, SOD3) found in the extracellular matrix, cytoplasm, mitochondrial intermembrane space, nucleus, and lysosomes. Another enzymatic system which is responsible for conversion of reduced glutathione (GSH) to oxidized glutathione (GSSG), catalyzed by glutathione peroxidase (GPX) [2]. Non enzymatic systems include chemical antioxidants like glutathione (GSH), α -tocopherol (vitamin E), and ascorbic acid (vitamin C). GSH act as a cosubstrate in the reduction of H_2O_2 by GPx. It might also react with oxygen-free radical directly, similarly, vitamins E and C also reduce oxygen-free radicals. They act by trapping hydroxyl radicals and other reactive radicals and thus break radical chain reactions and form new less reactive radicals [3]. Reactive oxygen species (ROS) are reported to be involved in different cellular processes ranging from apoptosis and necrosis to cell proliferation and carcinogenesis. Reports confirm the ECS (endocannabinoid system), may play an important role in the regulation of cellular redox homeostasis [4]. Endocannabinoids such as AEA are also known to mediate some of their cellular responses by targeting the non-selective cation channel TRPV1, whose activation has been linked to increased ROS production, AEA has also been reported to target the PPAR family of nuclear receptors, whose activation is known to induce the expression of antioxidant enzymes, including catalase and glutathione peroxidase 3 [5]. Thioredoxins (Trx), function as hydrogen donors to thioredoxin-dependent peroxide reductases. These have a Cys-Gly-Pro-Cys active site, which is indispensable for redox regulatory functions of thioredoxins. Two isoforms of Trx have been observed, these are Trx1 (expressed in the cytoplasm and the nucleus) and Trx2 (expressed in the mitochondria), which are very crucial for cell survival. This implicates its protective role against reactive oxygen species [6].

Cellular Redox Homeostasis is determined by the ability of a cell to maintain the balance between the magnitude of generated oxidative stress and the rate of its detoxification [7]. Maintaining the redox balance is important for proper function and responses of cells. Any disturbance in the redox homeostasis induces oxidative stress mediated signaling cascade that could lead to cell death or induce adaptive survival responses. Outcome of perturbation in the redox balance depends on the magnitude of oxidative stress induced inside the cell [8]. The intracellular "redox homeostasis" or "redox buffering" capacity is maintained primarily by glutathione (GSH oxidized / reduced) and thioredoxin (TRX oxidized/reduced) redox couples. GSH/GSSG ratio represents the major cellular redox buffer and it is therefore used as an indicator of the redox environment of the cell. GSH, Trx and glutaredoxin rectifies the thiol modifications due to oxidative stress. GSH reductase and Trx reductase reduces the GSSG and oxidized Trx at the expense of NADPH. The reducing nature of GSH and Trx is pivotal for the clearance of peroxides by peroxidase and peroxiredoxin [9]. Basal levels of reactive oxygen species (ROS) are endogenously produced in the mitochondria due to partial reduction of oxygen, inflammatory reactions and enzyme linked reactions viz. NAPH oxidase, Xanthine oxidase, cytochrome c oxidase [10]. Induction of mild oxidative stress activates redox sensitive pro-survival pathways like Nrf2 that protects against the oxidative damage. High oxidative stress leads to induction of apoptosis. Owing to their high reactivity, high levels of generated ROS react with the molecule including proteins, carbohydrates, lipids and DNA in the vicinity non-specifically (**Figure 1**).

This further results in impaired functioning of key cellular components directing the cell towards apoptosis [11]. However, generation of low levels of ROS acts as secondary messenger and activates numerous redox-sensitive pro-survival signaling pathways [12]. Spatio-temporal generation and regulation of ROS underlines their

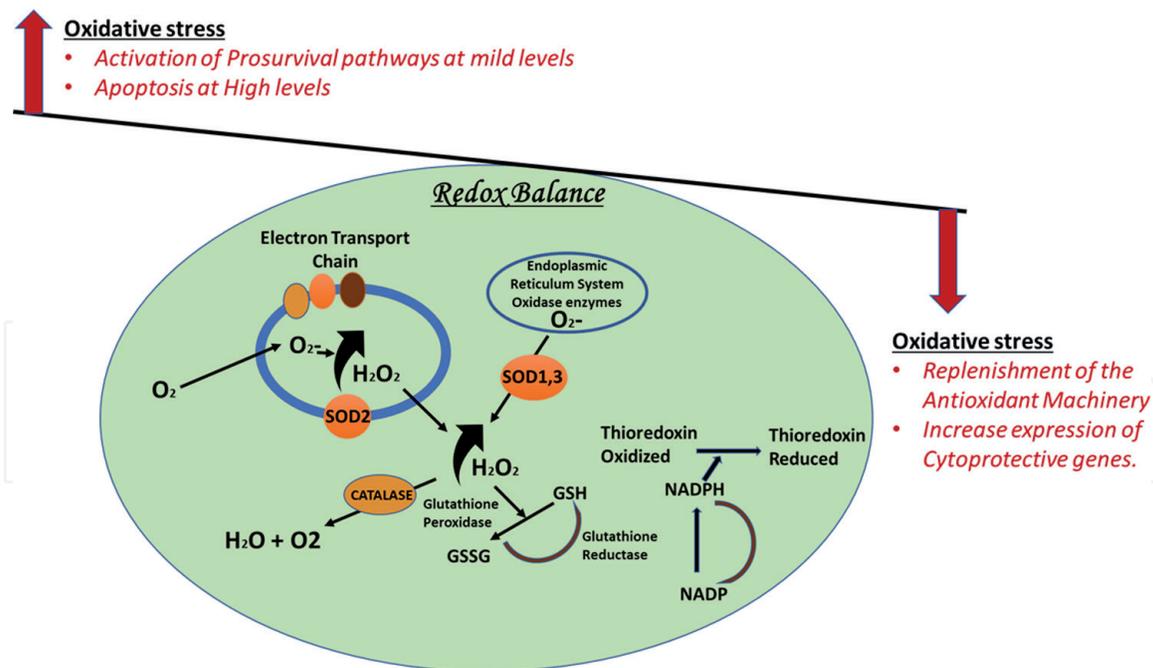


Figure 1. Cellular redox homeostasis: Electron transport chain in mitochondria, endoplasmic reticulum and oxidases enzymes like NADPH oxidases and xanthine oxidase induces the generation of superoxide and peroxide radicals. The increase in hydrogen peroxide elevates the redox balance towards oxidative stress. Spatial-temporal regulation of the generated oxidative stress determines the cellular fate in term of apoptosis or cytoprotection. Activation & expression of transcription factors leading to the replenishment of antioxidant and cytoprotective machinery of the cell leads to restoration of redox balance.

potential to contribute as secondary messenger from cell surface to the nucleus [13]. Scavenging of endogenous ROS can impair normal cellular response like production of cytokines and growth factors by T cells. Redox state of many proteins plays an important role during immune responses [14]. Critical cysteine residues present on proteins act as redox sensors and are prone to oxidation into sulfenic acids or disulphide formation or glutathionylation resulting in the modulation host immune responses [15]. The effect of oxidative stress on functions of these proteins depends on the concentration, duration and location of ROS generated inside the cell. A number of transcription factors and families have been identified which work in events of redox homeostasis or signaling, namely AhR, AP1, β -catenin, Egr-1, FOXO, HIF-1 α , p53, NF- κ B, Nrf2, Sp1, TTF. The present chapter is aimed at narrating the role of Nrf2 and NF- κ B in redox environment and their redox regulation. Nrf2 being the master regulator is further detailed as putative target with therapeutic potential under multiple clinical settings.

2. NF- κ B: redox sensitive transcription factor

NF- κ B plays an important role in regulating the immune and inflammatory response. NF- κ B is a ubiquitous transcription factor [16]. p52/p100, NF- κ B p50/p105, C-REL, RELA/p65 and RelB constitute the Nuclear factor- κ B (NF- κ B) family of transcription factors. These factors mediate the transcription of target genes by binding to a specific DNA element, κ B enhancer, as various hetero- or homo-dimers [17]. They also regulate the various biological responses such as immune responses, cell differentiation, cell proliferation, survival, stress response and inflammation. But the most studied and well-known function of NF- κ B is in the inflammation, regulating the pro inflammatory cytokines, activation, differentiation and effector functions of T cells [18]. NF- κ B has the ability to detect the stimuli such as infectious

agents, UV radiation, ROS, Tissue injury, lipopolysaccharide (LPS), and free radicals which activate NF- κ B [19]. The basic mechanism involves the tissue injury which activates NF- κ B, dissociates I κ B as a result of which the NF- κ B enters the cell nucleus and activate the DNA to enhance the inflammatory cytokines [20]. Regulation of NF- κ B activity is achieved through various post-translational modifications of the core components of the NF- κ B signaling pathways. There are two pathways by which NF- κ B is regulated; the canonical and the alternative pathway [21]. The canonical pathway is responsible for the installation of pro inflammatory cytokines, chemokines and other inflammatory mediators which directly engage into inflammation and act indirectly. Activation of the non-canonical NF- κ B pathway involves different signaling molecules and leads to the predominant activation of the p52/RelB dimer. An “alternative” NF- κ B pathway is activated by TNF-family cytokines—lymphotoxin b (TNFSF3) CD40 ligand (CD40L and TNFSF5), B cell activating factor (BAFF and TNFSF13B), and receptor activator of NF- κ B ligand (RANKL and TNFSF11).

2.1 The canonical pathway

The canonical pathway is provoked by the pro-inflammatory cytokines, ligands of varied immune receptors and involves the rapid and transient activation of I κ B kinase [22]. NF- κ B activity at sites of inflammation is associated with activation of the canonical pathway and RelA- or cRel-containing complexes [23]. In the pathway, NF- κ B/Rel proteins are tethered which are inhibited by I κ B proteins. Pro inflammatory cytokines, lipopolysaccharide, growth factors and antigen receptors activate the IKK complex (IKK β , IKK α and NEMO) [24]. The complex then phosphorylates I κ B proteins which lead to ubiquitination and proteasomal degradation, freeing NF- κ B/Rel complex. Active NF- κ B/Rel complex is further activated by post transcriptional modifications and translocate to the nucleus, where either alone or in combination with other transcription factors including AP-1, Ets and STAT and induce target gene expression [25].

2.2 The alternative pathway

NF- κ B2 p100/RelB complexes are inactive in cytoplasm. Signaling in LT β R, CD40, BR3 activate kinase NIK [26] which in turn activate IKK α complex that phosphorylate C terminal residue in NF- κ B2 p100 which leads to ubiquitination and proteasomal processing to NF- κ B2 p52 and translocate to nucleus to target gene expression [27]. The pathway regulates important aspects of immune functions, including lymphoid organ development, the cross-priming function of dendritic cells, B cell survival and germination center reactions, generation and maintenance of effector and memory T cells, antiviral innate immunity [28]. The pathway is responsible for inflammatory disease, kidney inflammation, metabolic inflammation and central nervous system inflammation [29]. Recent evidence suggests that NF- κ B also has a role in regulating the activation of inflammasomes. Dysregulated NF- κ B activation is a hallmark of chronic inflammatory diseases. Therefore, a better understanding of the mechanism that underlies NF- κ B activation and pro-inflammatory function is of great significance for therapeutic strategies in the treatment of inflammatory diseases.

3. Redox modulation of NF- κ B

Imbalance in redox state is redox modulation. The redox state of cells controls the activation and inhibition of NF- κ B, as in the state of oxidative stress that can both activate and inhibit NF- κ B by targeting the upstream kinases [30]. Activation

of NF- κ B by regular signaling is well known, however NF- κ B activation also depends on redox state of cells in three possible ways: (i) many NF- κ B -activating substances cause the production of reactive oxygen species (ROS) superoxide, H₂O₂, lipoxygenase products or act as oxidants on their own, (ii) NF- κ B activation can be caused by superoxide H₂O₂ or organic hydroperoxide in some cell lines when no physiological stimulation is present, and (iii) A wide range of NF- κ B inhibitors inhibit NF- κ B – activation and antioxidants that are chemically unrelated. These observations have led to a consensus that NF- κ B activation is related to some oxidative reaction. Molecules like thioredoxin, escalates the activity of NF- κ B to bind DNA under oxidative stress [31]. A component of dynein motor complex LC-8 also participates in redox regulation of NF- κ B. It activates NF- κ B on exposure of TNF α and results in ROS production which oxidizes LC-8 and its dissociation from I κ B α thus leading to NF- κ B activation. Reportedly, NF- κ B activation has anti-oxidant and pro-oxidant roles, the former involves the suppression of ROS accumulation, autophagy promotion, Inhibition of JNK activation and increased anti-oxidant targets whereas the pro-oxidant role includes the induction of pro-oxidation genes. One of the most important molecules in regulating redox modulation is hydrogen peroxide, it has been a question of debate, if H₂O₂ is involved in redox activation of NF- κ B. As indicated in literature, TNF α induced activation of NF- κ B mediated by H₂O₂. TNF α is a strong activator of NF- κ B, that induces superoxide formation in mitochondria. As in Wurzberg cells where H₂O₂ directly activates NF- κ B. The findings were found to be inefficient when lymphoblastoid cell lines, Jurkat cells showed no results of NF- κ B activation by H₂O₂ [31, 32]. Various exogenous and endogenous sources can enhance the redox reaction. Redox reactions play a huge role in inflammation specifically in lung inflammation where oxidative injury is most common due to its structure and function. ROS production is an immune response against inhaled pathogens and pollutants like cigarette smoke, automobile exhaust. Excess production of endogenous ROS leads to chronic inflammatory lung disease such as chronic obstructive pulmonary disease, asthma and pulmonary fibrosis. Oxidative stress produced by cigarette smoke activates NF- κ B by activating IKK complex which interferes with the chromatin modifications that escalate the transcription of pro-inflammatory genes [33].

4. Nrf2: the master regulator

The nuclear factor erythroid 2 (NFE2)-related factor 2 (Nrf2) is a member of the cap 'n' collar (CNC) subfamily of basic region leucine zipper (bZip) transcription factors including nuclear factor erythroid-derived 2 (NFE2) and NRF1, NRF2, and NRF3. There are seven conserved NRF2-ECH homology (Neh) domains within NRF2 gene, with different functions to control NRF2 transcriptional activity. The bZip in the Neh1 domain acts to activate gene transcription by forming dimer with small musculoaponeurotic fibrosarcoma proteins (sMAF). Neh2 domain mediates NRF2 ubiquitination and degradation as it contains ETGE and DLG motifs which act together with Kelch domain of Kelch-like-ECH-associated protein 1 (KEAP1) [34]. The Neh3-5 domains find their role as transcriptional activation domains, Neh6 domain works to mediate Nrf2 degradation in cells experiencing oxidative stress. Neh7 domain mediates interaction with retinoic X receptor alpha (RXR α), which represses Nrf2 activity. It is involved in the control of development of labial and mandibular segment of *Drosophila* by basic leucine zipper DNA binding domain (bZip) homeotic gene [35].

Removal of Nrf2 alters the defense machinery of the cell against oxidative stress. Knocking out Nrf2 has no effect on the mortality of the mice. Basal level expression

of Nrf2 in the cytoplasm ensures the production of cytoprotective proteins to exert normal physiological redox homeostasis [36]. Modulation in redox status is known to activate prosurvival redox sensitive Nrf2 pathway. Under normal condition Nrf2 is sequestered in cytoplasm by the inhibitor KEAP-1. Abrogation of KEAP-1 binding leads to translocation of Nrf2 to the nucleus mediated by nuclear localization signal. In nucleus Nrf2 forms a heterodimer with the co-transcription factor MAF. The heterodimers bind to the corresponding antioxidant response element and induces the expression of downstream cytoprotective and antioxidant enzymes [37]. The Nrf2 system is considered to be a major cellular defense mechanism against cellular oxidative stress. Nrf2 plays an important role in cellular defense and in improving the removal of ROS by activating downstream genes that encode phase II detoxifying enzymes and antioxidant enzymes, such as GCLM, NQO1, HMOX1, GPX, and glutathione S-transferases (GST) [38]. Nrf2 controls the expression of key components of the glutathione (GSH) and thioredoxin (TXN) antioxidant system, as well as enzymes involved in NADPH regeneration, ROS and xenobiotic detoxification, heme metabolism, thus playing a fundamental role in maintaining the redox homeostasis of the cell. Excessive ROS production causes oxidative stress to increase mitochondrial DNA damage, further promotes the activation of oncogenes or the inactivation of anti-oncogenes, which facilitates its tumorigenic signaling pathways and tumor progression. Nrf2/ARE pathway protects cells against oxidative stress via regulating the expression of Sestrin 2 gene as evident by monitoring the expression of downstream antioxidants. Sestrin 2 has strong antioxidant capacity and can provide cell with cytoprotective against various harmful stimuli. Sestrin blocks mTOR expression and mitigates the accumulation of ROS [39].

5. Redox regulation of Nrf2

Redox regulation underlines the cellular homeostasis. Regulation of redox sensitive transcription factors play a pivotal role in determining the cellular fate. Nrf2 being a master regulator is at the focal point of maintaining and regulating the cellular redox equilibrium. Perturbation in redox equilibrium is known to modulate the Nrf2 activation and hence effect the cellular fate [19]. Spatio-temporal generation of oxidative stress determines the graded activation of redox sensitive mediators. Mild oxidative stress is known to activate Nrf2 pathway and increases the cytoprotective proteins. Redox based activation of Nrf2 is attributed to the presence of more than 20 critical cysteine residues present in the KEAP-1 protein (**Figure 2**) [40]. 273 and cys288 have been shown as critical for abrogating KEAP-1 mediated inhibition of Nrf2. Mutation in these residues render activation of Nrf2 by inhibiting the Cul3-E3-KEAP-1 mediated degradation of Nrf2. The mutation did not had any effect on the detachment of the KEAP-1:Nrf2 complex, thus allowing the nuclear translocation of free Nrf2. Further, cys151 was also implicated in redox modulator-based activation of Nrf2 pathway. Perturbation in cellular redox status by prooxidants have been shown to induce cellular oxidative stress and increase the glutathionylation of proteins. 1,4 Naphthoquinone treatment induced glutathionylation of KEAP-1 for inducing Nrf2 pathway owing to its prooxidant nature. Apart from the cytoprotective nature of Nrf2, its role as a redox sensitive anti-inflammatory transcription factor has been well documented. Multiple reports have highlighted redox modulation based modulating role of Nrf2 in ameliorating immune-pathologies [41]. Nrf2 knockout mice exhibited increased bronchial inflammation, prolonged inflammation, high susceptibility for autoimmune syndrome, elevated lymphocyte proliferation and impaired redox homeostasis. Nrf2 dependent proteins including HO-1 inhibits the cytokine secretion, leukocyte

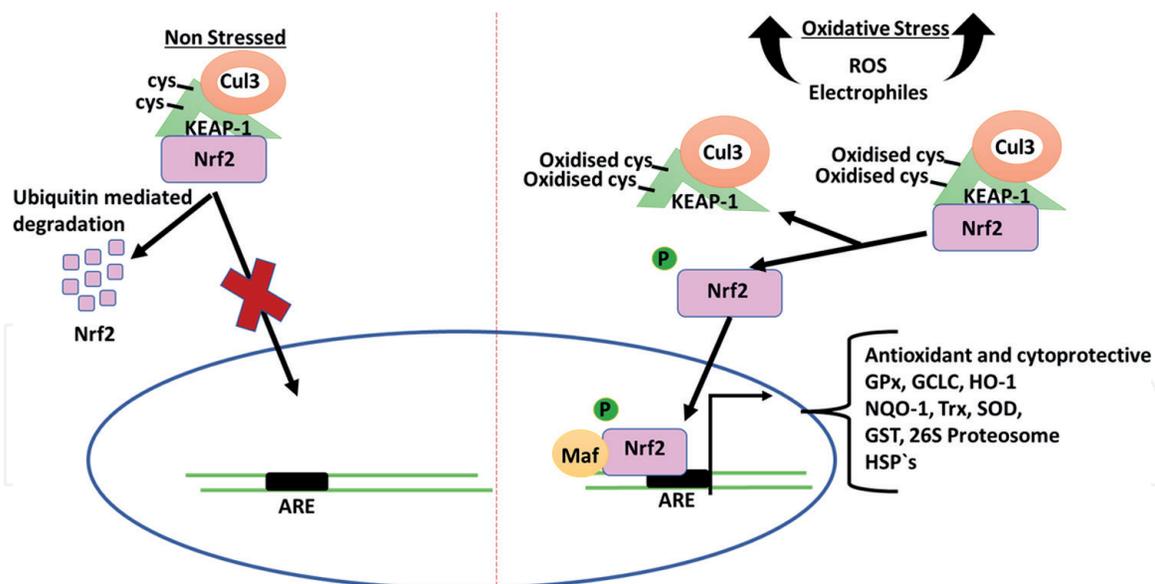


Figure 2. Redox regulation of Nrf2 pathway. Under non-stressed condition, Nrf2 is sequestered by its inhibitor KEAP-1 in the cytoplasm by inducing ubiquitin mediated Nrf2 degradation. Increase in ROS and electrophiles induces oxidative stress which results in oxidation of critical cysteine residues in the redox sensitive transcription factors. Cysteine residues of KEAP-1 are oxidized to release Nrf2 which translocates to the nucleus to express the downstream genes of antioxidant and cytoprotective potential. Thus, redox perturbation and tickling induces the Nrf2 pathway that act as master regulator for maintaining cellular redox equilibrium in turn governing the cellular fate.

migration, adhesion and suppressed LPS induced production of tumor necrosis factor- α (TNF- α) and nitric oxide (NO) in murine macrophages. Ablation in HO-1 protein increases the susceptibility towards autoimmune diseases [42].

6. Therapeutic potential of Nrf2

Excessive reactive oxygen species are threat to cells redox homeostasis which are major cause of oxidative stress leading to maladies like cellular dysfunction in aging, cardiovascular disease, renal dysfunction, diabetes, cancer is some to name a few. Antioxidant therapies play an important role in combating the progress of these diseases but the results are not satisfactory therefore there is an urgent need for a solution which activates endogenous antioxidant defenses. The redox-sensitive transcription factor NF-E2 related factor 2 (Nrf2) plays a significant role in synchronizing cellular antioxidant defenses and maintaining redox homeostasis [43]. Nrf2 is the “master regulator” of the antioxidant response, it modulates the expression of many genes, that include antioxidant enzymes, immune and inflammatory responses, tissue remodeling and fibrosis, carcinogenesis and metastasis. NRF2 activation by Keap1 binding is one of the major pathways that senses the oxidative stress, particularly there are Four reactive cysteine residues identified in Keap1 are most likely nominees for being the direct sensors of oxidative stress, various alternative mechanisms for Nrf2 activation were discovered, which are dependent upon kinase pathways, these include mitogen-activated protein kinases (MAPK), phosphatidylinositol-3 kinase and atypical protein kinase(s) C. Cell culture experiments report many compounds which show the ability to activate Nrf2. Large number of Nrf2 activators are principally naturally-occurring and plant-derived such as sulforaphane and curcumin and found in foods, but synthetic compounds have also shown to act as activators for instance bardoxolone methyl [44].

The Therapeutic potential of Nrf2 and its activators have been studied in various diseases. Some of them are as follows:

6.1 Diabetic nephropathy

It is the leading cause of chronic kidney disease, some mechanisms contribute to the onset and pathogenesis of diabetic nephropathy, including genetic and hemodynamic factors, oxidative stress, and cytokine signaling. Diabetes triggers oxidative stress through different ways, such as advanced glycation end-product accumulation and activation of polyol pathway, protein kinase C pathway, and renin angiotensin-aldosterone system. Loss or decrease in expression of SOD or glutathione in renal diseases are overcome by restoring and check the progression of disease. Nrf2 regulates expression of genes through ARE (antioxidant response elements) in their promoters to neutralize free radicals and accelerate removal of environmental toxins. Protective role of Nrf2 against renal damage has been demonstrated on streptozotocin induced diabetic rats, wherein it was shown that it slows the progression of diabetic nephropathy, Nrf2-mediated protection works through the negative regulation of TGF- β 1 and p21/WAF1Cip1 (p21) [45]. Reports suggest the Nrf2-dependent anti-oxidative and anti-inflammatory effects of digitoflavone in streptozotocin-induced diabetic nephropathy, *in vitro* using SV40-transformed mouse mesangial cells (SV40-Mes13), results showed that Digitoflavone activated Nrf2, reduced oxidative damage, inflammation, TGF- β 1 expression, extracellular matrix protein expression and mesangial cell hyperplasia [46]. Enhancement of NRF2 activity in the renal tubules considerably improves damage related to kidney injury and prevents its progression to chronic kidney disease (CKD) by reducing oxidative stress. KEAP1-NRF2 system along with Nrf2 activators have also been proposed to be a target for renal defense, wherein KEAP 1 inhibitors like CDDO-methyl ester, a synthetic triterpenoid are the main targets for the study as they work by inhibiting KEAP1-Nrf2 bonding which lets stabilized and free Nrf2 to translocate to nucleus and activate downstream genes [47]. Another strategy employed is suppressing the degradation of Nrf2 via inhibiting proteasome activity by inhibitors like minocycline, Increasing of Nrf2 concentrations within the cells have been found to be effective against the renal damage, reports suggest that Zinc helps elevating the Nrf2 protein level within the nucleus and upregulated the expression of Nrf2 downstream enzymes by encouraging inhibition of Nrf2 nuclear promoter Fyn mediated by Akt/GSK-3 β , Resveratrol and its analogue (polydatin) have reported to activated Nrf2/ARE pathway through upregulating Sirt1 (NAD-dependent histone deacetylase in the nucleus) in glomerular mesangial cell [48].

6.2 Cancer

Systematic studies of carcinogenesis specify an important role of endogenous oxidative damage to DNA, and an imbalance of cellular redox homeostasis that is balanced by elaborate defense and repair processes [49]. Pancreatic cancer is the most fatal diseases, it has very high rate of metastasis, Keap1-Nrf2 pathway is an emerging target for PC prevention and therapy. Certain modulators like UHRF1 (ubiquitin-like containing PHD and RING finger domains 1) is overexpressed in pancreatic cancer and are correlated to tumor growth. UHRF1 suppresses Keap1 expression by promoter methylation, this leads to Nrf2 activation. MBD 1 and p62 have been reported to inhibit ROS and promote tumor growth and drug resistance by inducing Nrf2 accumulation, nuclear translocation and activation. Nrf2 activation by a has also been found to inhibit PC cell growth and induce apoptosis by upregulating HO-1 [50]. The compound D3T (3*H*-1,2-dithiole-3-thione) has been shown to increase the nuclear accumulation of Nrf2, Honaucin A, natural marine-based compound, obtained from cyanobacteria, forms a covalent bond

with the sulfhydryl groups on KEAP1, resulting in the activation of Nrf2, phenol, polyphenol, or triterpenoid majorly form class of compounds that activate Nrf2. Sulforaphane is highly electrophilic molecule, it non-covalently binds to sulfhydryl groups of KEAP1 resulting in Nrf2 activation. Sulforaphane can also activate antioxidant response elements (AREs) associated with Nrf2. Micro RNAs (miRNAs) miR-141, miR-432-3p, miR-200a, have also shown to modulate the activities of KEAP1 and NRF2 in ovarian carcinoma cell lines, breast cancer, esophageal squamous cell carcinoma (ESCC), endometrial cancer tissues, miR-7, directly targeted KEAP1 mRNA in neuroblastoma SH-SY5Y cells, where it activated NRF2-dependent transcription of the antioxidant genes HMOX1 and GCLM [49]. Aberrant Nrf2 activation with in cancer cells may be due to somatic mutations within the Nrf2, KEAP1, or CUL3 genes, or the increase of KEAP1 interacting proteins, such as p62/Sqstm1 and p21, or it may be due to cysteine modification by oncometabolites such as fumarate all of the above activities may confer resistance to cancer cells and hence form the major targets for the cancer therapies. Nrf2 inducers are reported to hasten the detoxification of carcinogens (often electrophiles) from the cell and hence protect from chemical carcinogenesis Nrf2 inhibitors like brusatol work as protein synthesis inhibitors, ML385, a thiazole-indoline compound binds to Neh1. Therefore, Nrf2 inducers and Nrf2 inhibitors may function as anticancer drugs, with different effects on different targets, Nrf2 inducers work in order to protect normal cells from carcinogens, whereas Nrf2 inhibitors suppress the proliferation of cancer cells that have acquired aberrant Nrf2 activation or Nrf2 addiction [51]. Keap1-deficient mice showed upregulation of detoxifying enzymes, including GST and NQO1, and higher Nrf2 activation [52]. Various epigenetic regulations like hypermethylation, histone modifications control the expression of Nrf2 and hence may form targets to different therapeutic strategies [53].

6.3 Pulmonary fibrosis or lung injury and inflammation

Pulmonary fibrosis is a progressive and irreversible disease; it is characterized by an increase in differentiation and of fibroblasts to myofibroblasts and excessive accumulation of extracellular matrix in lung tissue. A study reports antifibrotic function of sulforaphane (SFN), an NRF2 activator, was largely dependent on a long noncoding RNA [54]. Therapeutic potential of thymoquinone (TQ) in bleomycin-induced lung fibrosis (BMILF) were also investigated and it was seen that it decreases expressions of Nrf2, Ho-1 and TGF- β . Nrf2/Ho-1 signaling pathway is a principal target for TQ protective effect against BMILF in rats [55]. The protective role of Nrf2 is mediated by PPAR γ in hypoxia-induced Acute Lung Injury (ALI). Reports reveal that overexpression of Brg1 increases Nrf2 activity and reduces ROS and inflammatory factors in lung tissues. In lipopolysaccharide (LPS)-induced lung inflammation the defensive role of the PI3K/Akt-dependent activation of the Nrf2-HO-1 pathway was revealed in mice treated with desoxyrhapontigenin. Nrf2 knockout resulted in a worsening of asthma symptoms. Protective role of Nrf2 in emphysema induced mice can be correlated by its activation in alveolar macrophages. The role of Nrf2 dysfunction in COPD may be the result of loss of DJ-1. DJ-1 overexpression activates Nrf2 and inhibits apoptosis of alveolar type II cells that are undergoing Cigarette smoking-induced oxidative stress and inflammatory response. DJ-1 induces the activation of Nrf2 and increases the expression of downstream antioxidant machinery to reduce the oxidative stress. The underlines anti-inflammatory effects are attributed to the expression of HO-1. These findings highlight the role of DJ-1 as putative target for cigarette smoking induced lung diseases [56].

6.4 Neurodegeneration

Neurodegenerative conditions may be results of various primary causes which include including expression of certain gene alleles, toxicant administration, aging, protein aggregation, proteasomal or autophagic dysfunction, inflammation, neuronal apoptosis, oxidative stress, mitochondrial dysfunction, and interactions between neurons and glia. The earliest degenerative condition to be associated with oxidative stress was aging. Some diseases characterized as neurodegenerative are Parkinson's disease, amyotrophic lateral sclerosis (ALS), and Alzheimer's disease, Huntington's disease, Friedrich's ataxia, multiple sclerosis, and stroke. Nrf2 activation provides neuroprotection against oxidative stressors and mitochondrial toxins, including hydrogen peroxide, tert-butyl hydroperoxide, 6-hydroxydopamine, 3-nitropropionic acid (3-NP), 1-methyl-4-phenylpyridinium (MPP), and rotenone [57]. In various studies conducted water derivative of artemisinin namely artesunate and lipid soluble derivative artemisinin, artemether both show to enhance the activation of Nrf2 via increasing its nuclear translocation and binding to downstream antioxidant response elements, as well as through suppressing ROS-dependent p38 MARK and NF- κ B pathways [58]. Report suggests that SFN, an isothiocyanate compound that occurs naturally and can be derived from cruciferous vegetables such as broccoli is capable of activating Nrf2, Results show that SFN is able to cross the blood brain barrier, activate Nrf2-dependent gene expression in the basal ganglia, eventually protecting nigral dopaminergic neurons from cell death induced by MPTP. Wide variety of bioactive compounds like resveratrol, curcumin, naphthazarin, genistein, and carnosic acid and berberin have been reported as Nrf2 activators that show positive effects in neurodegenerative disorders by protecting dorsal root ganglion (DRG) neurons from glucose-induced injury also by antioxidant activity in primary spinal cord astrocytes exposed to H₂O₂ [59].

7. Conclusion

Cellular redox equilibrium is pivotal for normal cellular functioning and responses. Impairment in regulation and maintenance of redox homeostasis underlines the pathogenesis of multiple associated diseases. Thus, identifying the key players in redox regulation is of prime interest. Nrf2 and NF- κ B are the two most pivotal and embroiled redox sensitive transcription factors that underlines the maintenance of redox balance. Thus, the two pathways are at the epicenter of investigation with clinical significance. The Nrf2 is the master regulator of cellular redox status. The Nrf2 and its dependent genes are responsible for cytoprotection, immunoregulation, maintaining cellular antioxidant levels, reducing drug toxicities etc. Presence of multiple critical cysteine residues in KEAP-1, inhibitor of Nrf2, renders redox sensitivity in activation of Nrf2 pathway. Aberrant expression and regulation of Nrf2 pathway has been implicated in various pathologies including cancer, diabetes, neurodegeneration etc. Multiple researchers have demonstrated the targeting of Nrf2 as key strategy to curb inflammation and associated disorders. Apart from Nrf2, another redox sensitive transcription factor is NF- κ B. Aberrant activation of NF- κ B pathway has been implicated in inflammation, immunity, differentiation, cell growth, tumorigenesis and apoptosis. NF- κ B also contains cysteine residues which act as sensors for redox modulation. Recent advances have highlighted the cross talk between Nrf2 and NF- κ B as putative target for strategic drug development. Further, in depth clinically relevant exploration of the cross talk is warranted. The triangulate interplay of cellular redox, Nrf2 and NF- κ B have immense potential to generate the therapeutic benefits via serving a putative target for discovering and developing novel drugs.

Conflict of interest

There is no actual or potential conflict of interest.

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