Oxidative Stress-Induced Molecular and Genetic Mechanisms in Human Health and Diseases

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Abstract

Oxidative stress is a common denominator in many inflammatory diseases. A number of genetic and molecular factors are known to regulate oxidative stress by modulating cellular redox imbalance. These events lead to molecular and biochemical changes within the cells resulting in a myriad of biological phenomena including cell growth, initiation and progression of inflammation, programmed cell death, and cellular senescence. Reactive oxygen species (ROS) are well known to act as important secondary messengers which regulate gene expression through signal transduction pathways especially the MAP kinase and NF-κB pathway. Additionally, other molecular cascades are also regulated by oxidants. These molecular and genetic alterations lead to several changes such as genetic mutations, mitochondrial stress, and changes in NADH/NAD^+ ratio that further augment and aggravate oxidative stress leading to health deterioration and disease development. Antioxidants, on the other hand, regulate molecular pathways and gene expression within cells as part of the cellular defense system by destroying ROS through enzymatic and nonenzymatic mechanisms. In the present chapter, we discuss the genetic and molecular mechanisms that regulate oxidative stress and play a crucial role in human health and diseases.

Keywords

Oxidative stress • Reactive oxygen species • MAP kinase • Gene regulation • Inflammation

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1 Introduction

Oxidative stress resulting from exposure to various stress conditions constantly impact human health. Oxidative stress can be caused by infections, exposure to chemical agents, xenobiotics, or radiations. Under stress conditions cells tend to produce reactive oxygen species (ROS) [1, 2]. ROS can be generated either from the extracellular environment or from within the cell's own biochemical events. Exogenous sources include γ and UV radiations, xenobiotics, pollutants, toxins and food; while endogenous sources include immune cells, ROS-producing enzymes, mitochondria-mediated metabolism, and metal toxicity. Increased levels of ROS promote deleterious actions in cells, with potential to cause dysfunction in the biochemical system and alteration in normal physiological processes. These deregulations lead to a wide variety of degenerative processes and cause diseases such as neoplastic transformations and cancer, diabetic complications, chronic inflammatory diseases such as rheumatoid arthritis, psoriatic arthritis and inflammatory bowel diseases, atherosclerosis, acute inflammatory responses, and neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease [3, 4]. ROS act as signal intermediates and messengers and play an important role in the propagation of molecular signal transduction and altered gene regulation. Contrary to this, antioxidative mechanisms in cells tend to maintain redox equilibrium by activating alternate genetic and molecular machinery. Cells promote defensive mechanisms in the presence of oxidative challenge to curb the deleterious effects produced by oxidants. In both the responses the cell's genetic and molecular machinery play an important role in regulating the final outcome. The molecular signaling pathways induced upon oxidant exposure ultimately lead to transmission of signals to the nucleus where it regulates the expression of target genes and promotes appropriate physiological response. There are several pathways that are sensitive to the presence and exposure of oxidants, the most prominent and well studied among them is the MAP kinase pathway that eventually activates redox-sensitive transcription factor such as NF-κB which in turn regulates the expression of various proinflammatory genes [5]. In this chapter, the genetic and molecular mechanisms with regard to oxidative stress response specifically the MAP kinase/NF-κB pathway as well as pathways involving antioxidant response genes have been discussed. In addition, enzymatic and nonenzymatic mechanisms of the antioxidants have also been discussed.

1.1 Mitogen-Activated Protein Kinases (MAPKs)

The MAP kinase pathway, evolutionarily conserved and unique to eukaryotes, is one of the most studied signal transduction pathways [6]. The MAP kinase signaling pathway has three major families of kinases: extracellular signal-regulated kinase (ERK1/2), p38 kinase, and c-Jun N-terminal kinases/stress-activated protein kinases (JNK/SAPK). MAP kinases comprise a group of serine/threonine kinases that are activated in response to various signaling stimulus, e.g., exposure to oxidants, pollutants, radiations, as well as inflammatory cytokines. It is a vital signaling cascade which controls the gene expression related to cell growth, proliferation, differentiation, apoptosis, and immune response. Oxidative stress-induced activation of MAP kinase has been studied in various types of cells including T lymphocytes, hepatocytes, cardiomyocytes, fibroblasts, epithelial cells, endothelial cells, smooth muscle cells, and pleural cells [7]. ROS activates the ERK pathway through growth factor receptors or by direct activation of the Ras, a GTP-binding molecule involved in signal transduction, leading to the sequential activation of Raf kinase, MEK1/2(MAP kinase kinase), and finally ERK1/2. In the case of JNK/SAPK pathway, ROS activates tumor necrosis factor (TNF) receptors or alternatively activates either MAP kinase kinase kinase (MEKK1) or ASK1 proteins, which then activate MKK4/7 (MAP kinase kinase) and finally JNK/SAPK. JNK/SAPK activates various downstream transcription factors such as Jun, activating transcription factor 2 (ATF-2), nuclear factor erythroid 2-related factor

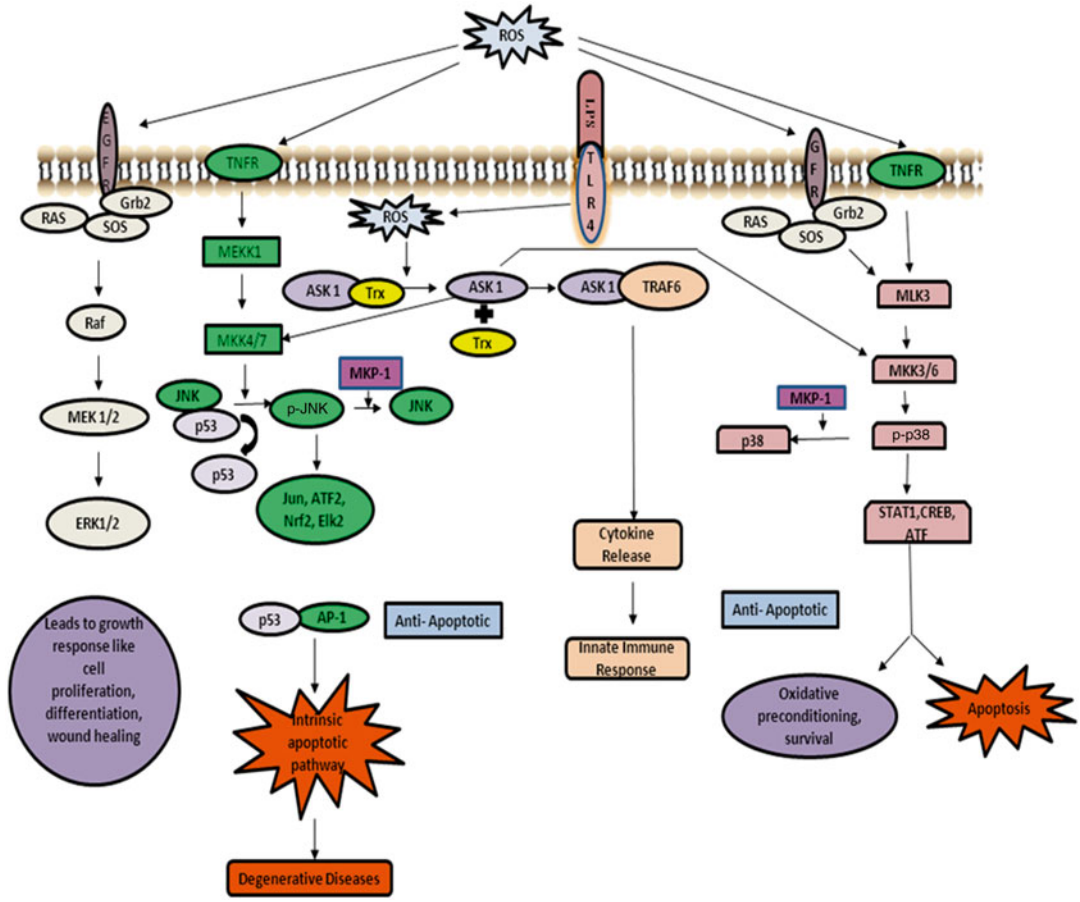

Fig. 1 The schematic diagram showing the activation of different MAPK pathways activated in response to exogenous and endogenous ROS produced during oxidative stress

(Nrf2), and Elk2 – a transcription factor belonging to the E-26 transformation-specific (Ets) family. The p38 pathway is activated either by the TNF receptor or EGF receptor activating downstream molecules such as cell division control protein 42 (cdc42), a GTP-binding protein, and Rac1 (Ras-related C3 botulinum toxin substrate 1), a GTP-binding protein of the Rho family, respectively, followed by activation of MKKKs and MKK3/6 which activate p38. The active p38 phosphorylates its corresponding target molecules such as transcription factors ATF and cAMP response element-binding protein (CREB) [7] (Fig. 1). Upon exposure to γ rays, X-ray, or UV irradiation, heat/osmotic shock and oxidative/nitrosative stress cells produce ROS and reactive nitrogen species (RNS) which act as key messengers and activate p38 and JNK/SAPK, which preferentially promote apoptosis [8]. Experimental evidences suggest the upregulation of MAP kinase pathways when exposed to oxidative agents such as H_2O_2 [9]. Endogenously produced through the process of respiratory burst, H_2O_2 induces the ERK1/2 pathway whereas exogenous source of H_2O_2 activates the p38 MAP kinase rather than ERK [10]. Hence, a balance among the three MAP kinase pathways is important to manifest a particular response by the cells to a specific type of stimulus. The ERK1/2 pathway mainly regulates cell proliferation and survival, whereas a decreased ERK and an increased JNK/SAPK lead to cell apoptosis [9]. The p38 could manifest either cell survival or cell death depending upon the nature of the stimulus.

1.2 Role of ROS-Induced MAP Kinases in Growth, Survival, and Homeostasis

ROS are mostly known for negative impacts on cells however, many studies have implicated the role of ROS in development and growth. Growth hormones, which play an important role in growth and development, are known to act through ROS. For example, PDGF induces ROS production in vascular smooth muscle cells and in lens epithelial cells [11], while TGFβ1 stimulates ROS production in lung fibroblast cells [12] and EGF in corneal epithelial cells [13] finally leading to promotion of growth in these cells. One of the important physiological functions in response to ROS observed in cells is to produce mitogenic stimuli and induce cell proliferation [13]. H_2O_2 is a type of ROS which possesses the ability to stimulate tyrosine phosphorylation of epidermal growth factor receptor (EGFR) and Src homology 2 domain-containing (SHC) adapter protein in a time-dependent manner. This is followed by the induction of a complex formation between EGFR and SHC-Grb2-SOS, thereby leading to Ras and ERK activation (Fig. 1). Hence, in vascular smooth muscle cells H_2O_2 mediates proliferation through EGFR activation via the MAP kinase pathway [14]. This suggests that besides their role in oxidant-induced cytotoxicity, MAPKs also mediate cell survival and homeostasis in the presence of growth factor-induced ROS leading to growth and development.

MAPKs play a vital role in immune response. ROS generated by phagocytic cells as part of an immune response against a pathogenic attack is an important aspect of survival and homeostasis strategy. MAPKs role in precipitating appropriate immune response is preceded by Toll-like receptor (TLR)-mediated pathogenic recognition. TLRs play a crucial role in the recognition of different pathogen-associated molecular patterns (PAMPs) such as lipids, proteins, lipoproteins, etc., of microorganisms and initiate an innate immune response followed by cytokine production. There are various types of TLRs (TLR1 to 11) depending upon specificity toward binding different ligands. Among these TLR4 is highly specific for gram-negative bacterial cell wall component, lipopolysaccharide (LPS). When bound to LPS, TLR4 recruits different adapter molecules such as TNF receptor-associated factor (TRAFF2/6), myeloid differentiation primary response gene 88 (MyD88), and Toll–interleukin 1 receptor (TIR) domain-containing adapter protein (TIRAP) and initiates a cascade of signals which eventually activate JNK and p38 MAP kinase pathway [15]. One of the mechanisms that underlie this innate immune response is ROS-mediated ASK1–MAP kinase activation. Apoptosis signal-regulating kinase 1 (ASK1) is a MAP kinase kinase kinase (MKKK), ubiquitously expressed in mammalian cells. LPS-induced TLR4 mediates ROS production through NADPH oxidase enzyme, associated with the membrane-bound TLR [16]. ROS then triggers the dissociation of thioredoxin (Trx) from ASK1 which is followed by the association of TRAF6 to ASK1. Binding of TRAF6 to ASK1 is followed by the activation of p38 MAP kinase leading to the transcription of cytokines and chemokines, which cause inflammation [17] (Fig. 1).

The role of p38 MAP kinase during oxidative stress has always been controversial. H_2O_2 challenged retinal pigment epithelial cells showed apoptosis through p38 MAP kinase pathway whereas the same cells when exposed to an oxidant *tert*-butyl hydroperoxide (t-BOOH), upon activation of p38 MAP kinase, portrayed a protective role [18]. The activation of p38 MAP pathway has been shown to play an important role in oxidative preconditioning, a process that protects cells upon subsequent exposure to oxidative agents for a long duration and at a higher dose [19]. Thus, ROS at low levels play a crucial role in maintaining a balanced redox state within the cells and also activate signal transduction pathways responsible for growth, differentiation, survival, and homeostasis. Further, insufficient levels of ROS can hinder the cells normal functioning such as growth and immune response, while overwhelming levels tend to propel the cell toward cell senescence and death.

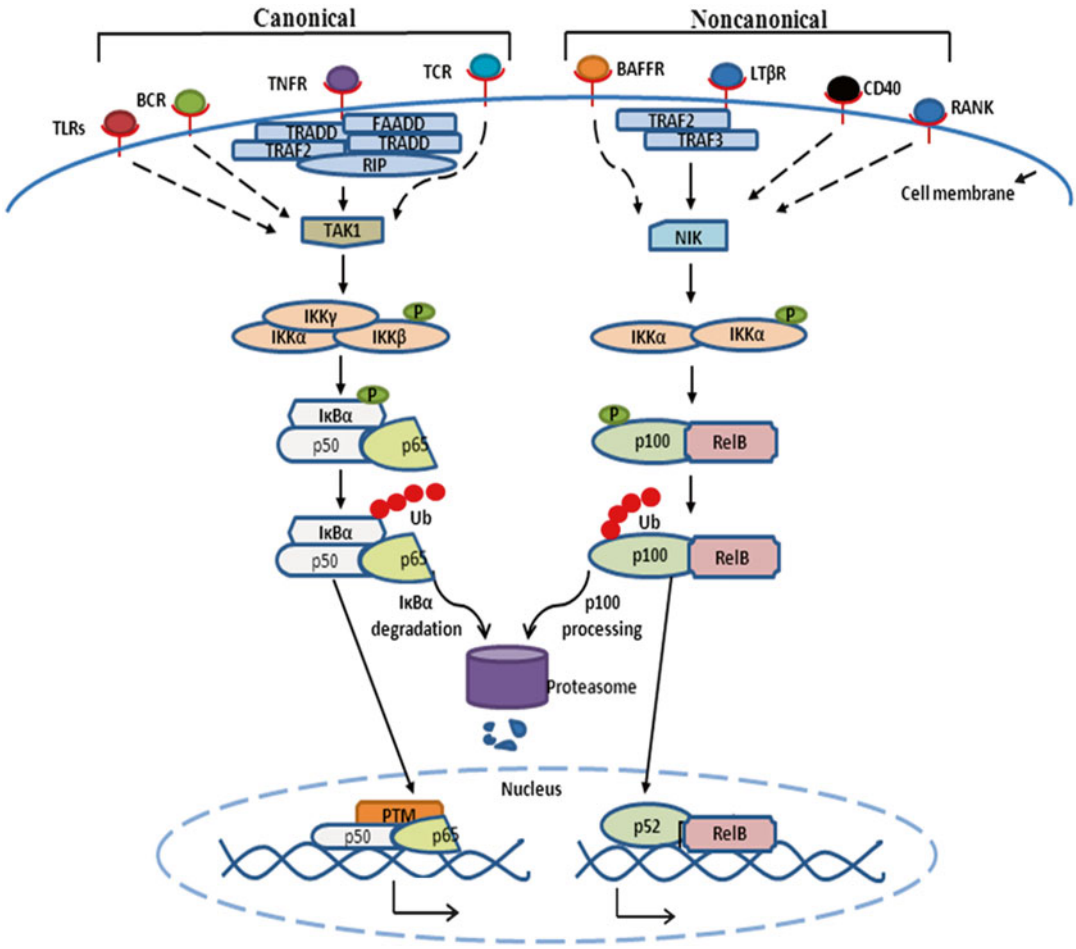

Fig. 2 Activation of canonical and noncanonical NF-κB pathways through different cell receptors

1.3 Role of Oxidative Stress-Induced MAP Kinases in Apoptosis

Apoptosis or programmed cell death is a crucial phenomenon required for development as well as a tool to promote cell death in the presence of incessant oxidative environment. As discussed above, the MAP kinase pathway is one of the main pathways through which oxidants regulate apoptosis through transduction of different apoptosis-inducing stimuli. ROS-induced protein oxidation in cells leads to induction of apoptosis through ASK1-mediated MAP kinase pathway. Upon activation ASK1 triggers the apoptotic response by phosphorylating JNK/SAPK and p38 MAP kinases, which subsequently phosphorylate and activate c-Jun transcription factor and ATF-2. The active c-Jun protein forms either a homodimer or a heterodimer with ATF-2 and forms active transcription factor AP-1 [8], which transcribes proinflammatory and apoptotic proteins. JNK/SAPK also regulates tumor-suppressor protein p53. Unphosphorylated JNK forms a complex with p53 leading to its ubiquitination and proteasomal degradation. Upon phosphorylation JNK phosphorylates p53 and stabilizes it by dissociating from the complex [20]. The active p53 then forms a complex with AP-1 and activates the intrinsic apoptotic pathway. AP-1-mediated pathway has been shown to induce apoptosis in mesangial cells [21]. On the contrary, the MAP kinase/

AP-1 pathway has also been shown to induce the expression of MAP kinase phosphatase-1 (MKP-1) in mesangial cells in a dose-dependent manner when exposed to H_2O_2. MKP-1, an oxidative stress-inducible enzyme, inactivates MAP kinases by dephosphorylating the specific tyrosine and threonine residues and protects against H_2O_2-induced apoptosis. All the three MAP kinases, ERK1/2, JNK/SAPK, and p38, need to be activated, and they cooperate to induce the expression of MKP-1. Although there are evidences of MKP-1 being regulated by pathways other than the MAP kinase, such as PI-3 kinase/Akt pathway in different cell types in response to H_2O_2 exposure, MAP kinase stands out to be a key regulator of the MKP-1, a vital antiapoptotic gene [22].

1.4 Role of Oxidative Stress-Induced MAP Kinases in Diseases

The cellular level of oxidants is an important aspect that determines the cells survival, proliferation, differentiation, or apoptosis. An imbalance in the redox status of cells is an important reason behind various types of human diseases. Oxidative stress has been implicated in various pathological conditions such as cardiovascular diseases, diabetes, neurological disorders, cancer, and age-related diseases. Heart diseases are a leading cause of death worldwide. The pathophysiology of cardiac hypertrophy is mediated by ASK1–MAP kinase pathway. Superoxide ions produced by angiotensin II (AngII) through its G protein-coupled receptor activates ASK1–MAPK pathway that leads to prominent heart diseases including cardiac hypertrophy, high blood pressure, and heart valve stenosis [23]. Alzheimer's disease, a neurodegenerative disease found among older people, is characterized by the presence of amyloid-β peptide, plaques, and neurofibrillary tangles. Amyloid-β deposition has been shown to activate ASK1 and subsequently JNK, leading to neuronal cell death [24]. Similarly, exposure to H_2O_2 and diethyl maleate, oxidative stress-inducing agents, activate p38, and ERK1/2 MAP kinase pathway leads to increase in damage of sensory neuron cells [9]. Further, diabetes, a metabolic disease affecting more than 300 million people worldwide who develop secondary diabetic complications such as retinopathy, nephropathy, and neuropathy, is caused by oxidative stress. Inflammation-induced ROS activates ASK1-mediated pathway leading to a proinflammatory condition followed by JNK activation that phosphorylates insulin receptor substrate 1(IRS1), a key mediator of insulin signaling. These steps lead to insulin resistance and an increase in blood sugar levels [25]. Hyperglycemic conditions established during diabetes lead to superoxide generation in endothelial cells in the arterial walls, promoting low-density lipoprotein (LDL) peroxidation [26]. The oxidized LDL alters the redox equilibrium within the cell followed by the activation of specific transcription factors AP-1 and NF-κB through the MAP kinase pathway, thereby causing an increase in the release of various growth factors like cytokines, which promote vascular smooth cell proliferation resulting in progressive plaque formation in the arterial walls [27]. This shows the interlink between diabetes and atherosclerosis and how oxidative stress and MAP kinase act as a common denominator [28].

The skin, being constantly exposed to oxidative agents such as UV radiations, pollutants, and pathogens, produces ROS exogenously and/or endogenously. A prolonged exposure to ROS could lead to a variety of skin disorders such as skin cancer, eczema, and psoriasis. Oxidants and ROS activate the redox signaling pathway including the MAP kinase, NF-κB, and Janus kinase/signal transducer and activator of transcription (JAK-STAT) signaling pathway which contribute toward the progression of skin diseases. For example, ERK1/2 levels have been shown to increase in lesional skin in comparison to the normal skin. Similarly, JNK and p38 MAPK pathways have been linked to over activation of TNF-α and keratinocyte hyper-proliferation by activating c-Jun in psoriasis [7].

MAP kinase pathway is an extremely complex signal transduction pathway involving numerous targets, which leads to different types of responses under oxidative stress. Under oxidative stress conditions all three MAP kinases, ERK1/2, JNK/

SAPK, and p38, are activated and cause oxidative injury and pathogenesis. Mostly ERK1/2 and JNK are associated with antiapoptotic response and lead to cell proliferation and differentiation, whereas p38 MAP kinase is linked with proapoptotic action. Thus, the MAP kinase pathway is an important oxidative stress-stimulated cascade that can be regulated and controlled by the presence of specific antioxidant agents and serves as important molecular target to prevent or treat oxidative stress-induced pathogenesis [9].

1.5 Role of Oxidative Stress-Induced MAP Kinases in Wound Healing and Repair

Signal transduction pathways are also implicated in wound healing and repair subsequent to oxidative injury and damage. Wound healing involves various important phases such as inflammation, cell proliferation, cell migration, and tissue remodeling. Different immune cells including macrophages and neutrophils get recruited in the wound site where they produce ROS which mediates the release of cytokines to protect the organism against the invading pathogen. Several studies demonstrate that inflammation caused by wound results in the release of H_2O_2, which activates p38 and MEK pathway leading to cytoplasmic translocation and active release of high-mobility group protein B1 (HMGB1) in monocytes/macrophages [29]. HMGB1 is a nuclear protein released passively in damaged cells. This protein triggers inflammation, attracts inflammatory cells, recruits stem cells, and promotes their proliferation ultimately leading to tissue repair [30]. Reepithelialization or cell migration of proliferated wound-related cells to the damaged site is of utmost importance. The extracellular matrix of the cells required for reepithelialization is degraded by matrix metalloproteinase (MMPs). A redox-dependent MMP production is observed during wound healing [31]. MMPs expression is activated by JNK through the Ets or AP-1 transcription factor or through the activation of NOX-4 (NADPH oxidase family), an enzyme that produces large amounts of ROS and activates MAPK and NF-κB in the downstream [32]. An apt example for wound healing is corneal wound healing. Cornea, the outermost transparent covering of the eye, is under constant exposure of UV-light, chemicals, bacteria, and fungus which injure or damage it. In a study, intracellular ROS produced in human corneal epithelial cells, upon exposure to epidermal growth factor, stimulated cell proliferation, adhesion, migration, and corneal surface wound healing [13]. In another study, the initial oxidative injury led to the release of keratinocyte growth factor and hepatocyte growth factor which activated p44/42 MAPK and p38 MAP kinase pathways leading to initiation of cell migration and proliferation that resulted in corneal wound healing [33].

Besides the different roles of the MAP kinase pathway during oxidative stress, as discussed in preceding sections the MAP kinase pathway also plays an important role in the antioxidative balance during oxidative stress. Homeostasis and redox balance within the cells need to be maintained by establishing an intricate equilibrium between oxidant production and destruction. The cells possess the ability to scavenge or quench the excess ROS produced within the cell by activating various antioxidant enzymes. The JNK and p38 pathways are known to activate an important transcription factor nuclear factor erythroid 2-related factor (Nrf2). Nrf2 is kept in an inactive state by interaction with the actin-anchored protein Kelch ECH-associating protein 1 (Keap 1) [34]. However, in oxidative stress Nrf2 is released and translocated into the nucleus where they bind to antioxidant response elements for the induction of cellular defense genes which include glutathione S-transferase, glutathione reductase, glutathione peroxidase, heme-oxygenase and NADPH quinone oxidoreductase-1. These gene products help in maintaining the redox balance by ROS disruption/quenching.

The identification of oxidative stress-induced molecular cascade has led to the investigation of various modulators, which may become important drug molecules against oxidative stress-induced diseases. Several phytochemicals possess the ability to ameliorate the oxidative damage caused by ROS. They act as exogenous

antioxidants and mediate their action through modulating oxidative stress-induced signal transduction pathways. For example, phytochemicals such as oleanolic acid has been shown to activate ERK and JNK pathway followed by activation of transcription factor Nrf2 [35]. Nrf2 regulates the levels of thioredoxin peroxidase, an H_2O_2-scavenging enzyme, and heme-oxygenase 1 (HO-1), a stress-inducible antioxidant enzyme localized in non-neural tissues, plays a key role in defense against inflammation and oxidative injury [36]. Quercetin, an antioxidant, showed Nrf2-induced expression of HO-1 in esophageal epithelial cells through the activation of MAP kinase and PI-3K pathways [37].

2 NF-κB Pathway

NF-κB, a redox-sensitive transcription factor, coordinates the different regulators of immunity, inflammation, cell survival, proliferation, and development [38]. Dysregulation of NF-κB activity is associated with inflammatory disorders, metabolic and autoimmune diseases, as well as cancer [39]. The NF-κB or Rel family of transcription factors is a downstream target of oxidative stress-induced MAPK pathway that is activated due to bacterial and viral infection, inflammatory cytokines, and upon ligand binding of antigen receptors, which are the sources of ROS. This suggests that NF-κB plays a crucial role in orchestrating immune and inflammatory response through regulation of different oxidant and antioxidant systems. The active NF-κB is a dimer made from monomer units p50, p52, p65 (Rel A), Rel B, and c-Rel proteins [5]. The members of the NF-κB family have a conserved region which is known as NF-κB/Rel/dorsal region or Rel homology domain. This conserved region spans the first 300 N-terminal amino acids and comprises three different regions such as a nuclear localization sequence, a DNA-binding domain, and a dimerization region.

In eukaryotes two alternates NF-κB-activating pathways occur: canonical and noncanonical pathways. Most of the NF-κB-activating physiological stimuli for instance signals from cytokine receptors, such as tumor necrosis factor receptor (TNFR) and interleukin-1 receptor (IL-1R), and antigen receptors such as B cell receptor (BCR), T cell receptor (TCR), and Toll-like receptor 4 (TLR4) induce the canonical pathway. The canonical pathway is characterized by the activation of IκB kinase (IKKβ) and IKKγ/NF-κB essential modulator (NEMO) cascade that leads to phosphorylation of IκBα, which then degrades and facilitates nuclear translocation of p65-containing heterodimer which transcribes target genes. The noncanonical pathway relies on IKKα-mediated phosphorylation of p100 associated with RelB, which leads to processing of p100 and the formation of p52–RelB complex. Specific members of the TNF cytokine family such as CD40 ligand, B cell-activating factor belonging to the TNF family (BAFF), receptor activator for nuclear factor κB (RANK) [40], and lymphotoxin-β2 (LT-β) induce noncanonical NF-κB signaling, although they may also regulate canonical pathway [41]. Different members of TNFR-associated factors (TRAF), TNF receptor-associated death domain (TRADD), and Fas-associated protein with death domain (FADD) adaptor proteins recruited at the intracellular portion of different receptors may activate both canonical and noncanonical signal transduction pathways [39] (Fig. 2).

In unstimulated healthy cells IκB, an NF-κB inhibitory protein which includes IκBα, IκBβ, IκBγ, and probably B cell lymphoma-3 (Bcl-3) is found bounded to a nuclear localization sequence of NF-κB and retains it in an inactive form within the cytosol [5]. ROS, generated by different oxidative mechanisms transduces hyperphosphorylation of IκB, which leads to its degradation by 26s proteasome complex via ubiquitination. After degradation of IκB, NF-κB proteins dimerize and translocate into the nucleus and transcribe various target genes.

2.1 Role of Oxidative Stress in NF-κB Pathway Activation

An antioxidant system activated through NF-κB can be expected to regulate the redox state when

the cell is under oxidative stress. There are two genes that could be induced by NF-κB, inducible Mn-dependent mitochondrial form of superoxide dismutase (MnSOD). MnSOD is expressed after stimulation of cells with TNF, LPS, and PMA. The second one is thioredoxin, which is induced by viral transactivator Tax through NF-κB [42].

ROS have paradoxical effects on regulation of NF-κB. A moderate increase in ROS leads to the activation of NF-κB, while severe increase inactivates NF-κB that could result in cell death. Moderately increased ROS-mediated activation of NF-κB increases cell survival by enhancing (a) expression of antiapoptotic Bcl-2 family members, such as Bcl-xL and A1/Bfl-1; (b) caspase inhibitors, such as IAPs; (c) $FLIP_L$, the active homologue of caspase-8; (d) GADD45, which inhibits JNK-mediated cell death; (e) TRAF1, TNF receptor-associated factor; and (f) antioxidants such as MnSOD and ferritin heavy chain (FHC) [43, 38]. The DNA-binding activity of NF-κB was found to increase with age, which reflects increased oxidative stress in aged tissues and cells [44].

The direct evidence that ROS regulates NF-κB has come from the exposure of different cells to H_2O_2 such as Wurzburg subclone of T cells, human breast mammary epithelial cell (MCF-7), skeletal muscle myotubes L6, and 70Z/3 pre-B cells and HeLa cells [45, 46]; however, some other studies could not detect NF-κB activation by H_2O_2 in HeLa cells [45].It indicates that NF-κB activation may be cell type specific [5]. Various indirect evidences also suggest a role of ROS as a common and critical intermediate for NF-κB activation. This is based on the fact that antioxidants such as butylated hydroxyanisole (BHA) and pyrrolidine dithiocarbamate (PDTC) as well as overexpression of antioxidant enzymes including SOD, thioredoxin, and GSH peroxidase inhibit/prevent NF-κB activation, although the extent of inhibition also depends on type of cell and stimulus [5].

Even though IκB degradation is one of the phenomena observed during oxidative stress, oxygen radicals have the capability to covalently modify several amino acids in proteins such as, cysteine, methionine, and histidine residues. Thus, direct oxidative damage can also cause dissociation of the NF-κB–IκB complex [42]. Further, few compounds are generated upon lipid peroxidation, such as malondialdehyde or 4-hydroxynonenol, that could covalently alter and dissociate the NF-κB–IκB complex [42]. However, much work is needed to confirm molecules and steps that are exactly responsible for oxidative stress-induced damage. Some studies suggest activation of IKK as the key regulatory step in NF-κB activation by oxidative stress and proinflammatory stimuli (TNF, IL-1, LPS, and ds-RNA). Thus, directly or indirectly ROS play a general signaling role in NF-κB activation [45].

2.2 Role of Oxidative Stress in NF-κB-Mediated Inflammatory Gene Induction

Inflammatory cytokines and chemokines promote NF-κB activation. These inflammatory mediators together with antimicrobial molecules and ROS cooperate to kill pathogens, eliminate infection, and remove dead cells. The end of inflammation is vital for maintaining good health while overwhelming inflammation is associated with various inflammatory diseases like rheumatoid arthritis (RA), type 2 diabetes, and atherosclerosis [47].

In the development of RA, the activation of NF-κB-dependent genes plays a key role. The receptor activator of NF-κB [40], CD40, B cell-activating factor, lymphotoxin β receptor, and TLRs are found to be upregulated in RA [40, 48]. Evidences show involvement of free radicals and NF-κB in the destruction of β cells and disease progression of insulin-dependent diabetes mellitus or type I diabetes [49]. Pancreas-specific ROS production plays a critical role in the autoimmune or inflammatory response by activating NF-κB [51]. Proinflammatory cytokines such as IL-1 have been observed to activate NF-κB in rat insulinoma cell lines [50]. The NF-κB pathway also seems to be involved in the insulin resistance of type II diabetes [51]. These evidences indicate that NF-κB is crucial in the development of

varied inflammatory pathologies and thus could be an important target in designing therapeutic approaches. Indeed, targeted disruption of IKK-β or salicylate inhibition of NF-κB has been shown to improve insulin sensitivity and reduction of obesity in mice [52].

3 Role of Oxidative Stress in Gene Regulation

Oxidative stress, an important modulator of cellular redox status, regulates the expression of proinflammatory/prooxidant genes as well as anti-inflammatory/antioxidative genes. The fine balance and eventual tilt between these two seemingly opposite mechanisms determine the fate of cells. As discussed in preceding sections, oxidative stress stimulates a molecular cascade that activates transcription factors, which transcribe various target genes including cytokines, chemokines, growth factors, and other inflammatory marker genes. It is therefore evident that gene expression is finely regulated in and by oxidative stress. The variation in oxidative status of cells alters the cellular environment, which affects transcription factors, and ultimately gene expression is affected.

Binding of transcription factors to a specific region of negatively charged DNA sequences requires accumulation of positively charged amino acids, resulting in stabilization of deprotonated thiol groups. In the presence of ROS, thiol residues such as cysteine get oxidized affecting DNA-binding efficiency. The signaling proteins behave differently to oxidation, depending upon the level of cysteine moiety in the cells during oxidative stress and intensity of oxidative stress. The signaling cascade of NF-kB and activator protein (AP-1), leading to gene regulation of glutathione reductase, serves as an example of oxidation of conserved cysteine groups, which is a reversible phenomenon [53].

Some of the gene products also turn on the transcription of various detoxifying enzymes and proteins such as glutathione S-transferase (GST) and NADPH quinone reductase that act as an antioxidant system during oxidative stress [54]. The cis-acting antioxidant response elements [55], responsible for inducible as well as constitutive gene expression of these two enzymes, mediate their transcriptional activation and are induced by sulfhydryl groups containing compounds such as diethyl maleate, isothiocyanates, and dithiothiones [56]. Similarly, oxidative stress also affects other transcription factors such as nuclear factor 1 (NF1), Sp1, and p53 NF1, activator of DNA replication, has a DNA-binding domain and a transactivation domain. The presence of oxidative stress affects cysteine residues of DNA-binding domain which hampers its DNA-binding activity through mutation to serine residues [54]. Regulation of redox imbalance by glutaredoxine or thioltransferase prevents mutation of its cysteine residues [54]. Sp1 is a ubiquitous transcription factor, rich in GC content, and has zinc finger motifs, which are crucial for DNA-binding activity. A decrease in glutathione level and increase in oxidants such as H_2O_2, represses the activity of Sp1-driven genes. Oxidative stress also represses the function of p53 by modulating its conformation as shown by decreased reporter gene expression [54, 57]. PDTC, which acts as prooxidant, increases the intracellular level of redox-active copper that downregulates p53. This would prevent the activation of p53 by stimuli such as UV or temperature shift, which are known to activate PDTC during oxidative stress. Oxidation of cysteine residue in p53 also inhibits or alters expression of apoptotic and DNA repair genes that leads to carcinogenesis [57].

The cytochrome P450 (CYP) genes encode for ubiquitous enzymes responsible for metabolism of xenobiotics. The NF1 binding site, located on CYP promoter, is the target for oxidation during redox imbalance. ROS is known to downregulate the isoforms of CYP, e.g., CYP1A1 by inflammatory cytokines, due to depletion of glutathione [54]. A study by Morel and Barouki [58] reported that increased TNF-α mRNA during oxidative stress downregulates this isoform of CYP through redox-sensitive mechanism involving NF1. This could also affect the NF1 and DNA interaction which in turn alters the redox-sensitive gene transcription [58].

Oxidative stress-induced alteration in gene expression is a well known cause of age-related disorders, e.g., Alzheimer's disease (AD) [59]. Various superoxide anion-producing enzymes such as NADPH oxidase (NOX) and xanthine oxidase (XO) along with mitochondrial dysfunction are major players in the oxidative stress-induced neurodegenerative disease. *NOX*, present in microglial cells, neurons, and astrocytes get activated by cytosolic subunit of G protein Ras. After activation, NOX translocates to the membrane and forms active NAPDH oxidase complex [16, 54]. The enzyme transfers protons across the membrane through anion channel to compensate the charges across the membrane and results in the formation of superoxide radicals. In astrocytes, amyloid β, an oxidant and inducer of NADPH oxidase, also activates the expression of *NOX* genes including *NOX1*, *NOX2*, and *NOX3* through CD36 receptor. The increased expression of NOX isoforms leads to an enhanced oxidative stress which depolarizes the mitochondrial membrane and induces opening of mitochondrial pore permeability and activates apoptosis that results in neuronal degeneration. Apart from neuronal death, aggregation of amyloid β protein leads to hyperphosphorylation of tau (τ) protein which is a primary or early stage biomarker in AD. A neuroprotective hormone melatonin acts as an antioxidant for NADPH enzyme complex and prevents the formation of amyloid β protein aggregation [60].

Oxidative stress-induced mitochondrial dysfunction, loss of function of DJ-1 gene, and A53T α-synuclein protein aggregation have been implicated in the pathogenesis of Parkinson's disease [61, 62]. DJ-1, a neuroprotective molecule, has an antioxidative mechanism in neurons. It increases the synthesis of glutathione, cellular redox buffer system, by regulating a rate-limiting enzyme glutathione–cysteine ligase (GCL). However, elevated level of ROS may cause allelic mutations in DJ-1 gene from cysteine to serine residues, leading to altered gene expression and progression of pathogenesis [62]. Additionally, under stress a nonfunctional GCL promotes A53T α-synuclein protein aggregation in dopamine neuronal cells which are responsible for cell death [60]. Glutathione improves survival of primary dopamine neurons and prevents dopamine-induced neuronal cell death [61, 62]. Although implicated in neuroinflammation, ischemia, age-related neurodegeneration, and Parkinson's disease, a detailed mechanism involving DJ-1/PARK7 gene function for ROS-mediated pathogenesis remains unclear [61, 63]. A better understanding of this pathway would be helpful in devising novel neuroprotective and anti-neurodegenerative therapy.

4 Conclusion

The emerging concepts regarding ROS and its effects on cellular physiology have gained enormous attention. This chapter focused on the molecular and genetic mechanisms and the different responses manifested by the cell when exposed to ROS. Deleterious effects of oxidative stress or the resultant antioxidant response to the same depend on the nature of the agents causing oxidative stress, their dosage and duration of exposure, and the signal transduction pathway activated resulting in specific transcription factor activation. Hence, it becomes difficult to determine the exact mechanism underlying ROS-induced stress at the molecular level. Nonetheless, several pathways have been identified, which get activated in response to ROS, especially the MAP kinase and NF-κB pathway. Many studies have implicated different upstream mediators that activate NF-κB, but how exactly cytosolic NF-κB complex senses oxidative stress is not well understood. Our knowledge is far from complete when it comes to identifying the precise upstream and downstream regulatory molecules in the oxidative stress-induced pathway and its regulation through gene expression. Future studies are required to clearly understand the molecular and genetic mechanism(s) that regulates oxidative stress-induced pathogenesis, which will lay the foundation for better preventive and therapeutic approaches for human health and diseases.

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