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Invited Review

Canonical and non-canonical mechanisms of Nrf2 activation

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ABSTRACT

Nuclear Factor Erythroid 2-related factor 2 (Nrf2) is a transcription factor that regulates the expression of genes involved in the metabolism, immune response, cellular proliferation, and other processes; however, the attention has been focused on the study of its ability to induce the expression of proteins involved in the antioxidant defense. Nrf2 is mainly regulated by Kelch-like ECH-associated protein 1 (Keap1), an adapter substrate of Cullin 3 (Cul3) ubiquitin E3 ligase complex. Keap1 represses Nrf2 activity in the cytoplasm by its sequestering, ubiquitination and proteosomal degradation. Nrf2 activation, through the canonical mechanism, is carried out by electrophilic compounds and oxidative stress where some cysteine residues in Keap1 are oxidized, resulting in a decrease in Nrf2 ubiquitination and an increase in its nuclear translocation and activation. In the nucleus, Nrf2 induces a variety of genes involved in the antioxidant defense. Recently a new mechanism of Nrf2 activation has been described, called the non-canonical pathway, where proteins such as p62, p21, dipeptidyl peptidase III (DPP3), wilms tumor gene on X chromosome (*WTX*) and others are able to disrupt the Nrf2-Keap1 complex, by direct interaction with Keap1 decreasing Nrf2 ubiquitination and increasing its nuclear translocation and activation. In this review, the regulatory mechanisms involved in both canonical and non-canonical Nrf2 activation are discussed.

1. Introduction

Oxygen is required in normal cellular functions such as energy metabolism in most eukaryotic organisms; part of this oxygen is partially reduced to the superoxide anion and subsequently to other reactive oxygen species (ROS) such as hydrogen peroxide and hydroxyl radical. In metabolism, the reactive nitrogen species (RNS), such as peroxynitrite and nitric oxide, are also produced. ROS and RNS, at low cellular concentration, are implicated in cellular signaling process, however an increase in these species leads to an oxidative stress state in the cells, inducing cellular damage and death [1]. Cells have developed a response called the phase 2 response, where a set of genes regulated by the Nuclear Factor Erythroid 2-related factor 2 (Nrf2), are involved in the defense against oxidative stress [2].

Nrf2 is considered the master regulator against oxidative stress. In homeostatic conditions, Nrf2 levels and its activation are controlled mainly by Kelch-like ECH-associated protein 1 (Keap1). Nevertheless, in an oxidative stress state or in the presence of electrophilic compounds, Nrf2 is activated, inducing the expression of its target genes, which are involved in cell protection. This mechanism of Nrf2 activation is known as the canonical mechanism. Recently, a new mechanism known as non-canonical has been described, where the activation of Nrf2 is carried out by Keap1-Nrf2 complex disruption by some proteins such as p62, DPP3, WTX, PALB2, p21 and BRCA1 [3]. In this article, we describe the canonical and non-canonical mechanism of Nrf2 activation and some peptides and drugs involved in the non-canonical activation as potential pharmacological targets.

Abbreviations: AhR, aryl hydrocarbon receptor; ARNT, aryl hydrocarbon receptor nuclear translocator; ATF4, activating transcription factor 4; Atg5, autophagy protein 5; Atg7, autophagy protein 7; Bach, BTB and CNC homology; CBP, CREB binding protein; CHD6, chromodomain-helicase-DNA-binding protein 6; CRIF1, CR6-interacting factor 1; Crm1, chromosomal maintenance 1; Hrd1, ERAD-asociated E3 ubiquitin-protein ligase HRD1; JDP2, c-Jun dimerization protein 2; LPS, lipopolysaccharide; Maf, musculoaponeurotic fibrosarcoma; MEF2D, myocyte enhancer factor 2D; Miro2, mitochondrial Rho GTPase 2; Neh, Nrf2-ECH-homology; NES, nuclear export signal; NLS, nuclear localization signal; PPARγ, peroxisome proliferator activated receptor γ; RAC3, receptor-associated coactivator 3; Rbx1, RING box protein 1; Sp-1, specificity protein-1; TAK1, transforming growth factor beta-activated kinase 1; UBNX7, UBX domain-containing protein 7; UFD1/NPL4, ER-associated degradation protein 1/ Nuclear protein localization protein 4 homolog; WDR23, WD repeat protein; XRE, xenobiotic response elements

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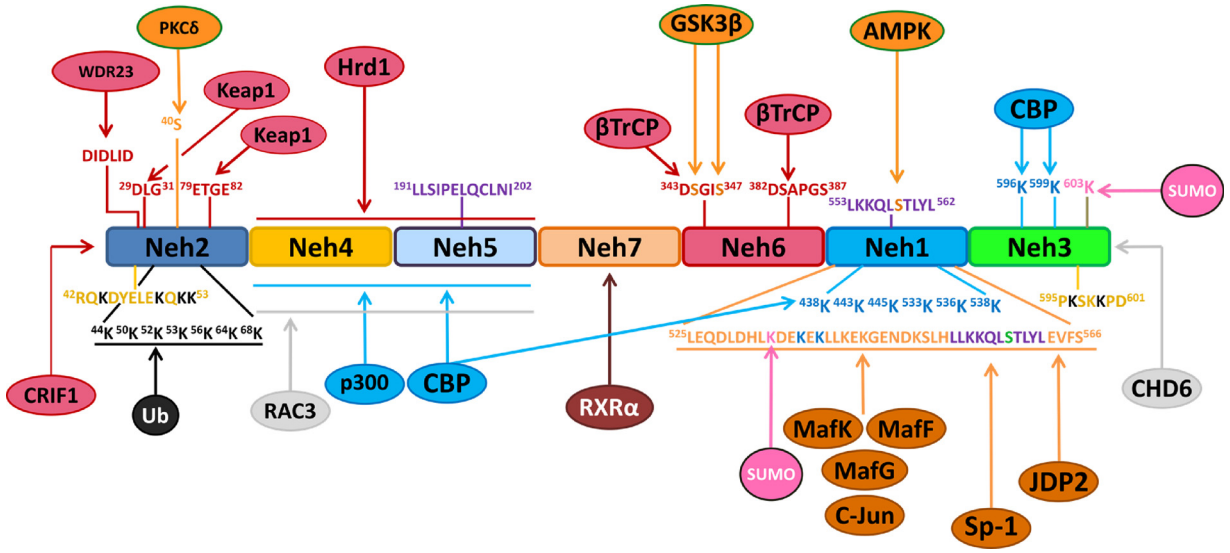

Fig. 1. Nrf2 protein structure from *Homo sapiens* (isoform 1). Nrf2 protein possesses 7 Neh domains (Neh1 to Neh7) in its structure. Neh1 domain contains bZip region (amino acid sequences between 525 and 566, on the bottom) through which it dimerizes with small Maf proteins (MafK, MafF and MafG) and other transcription factors (c-Jun, Sp-1 and JDP2). Furthermore it contains 6 Lys residues that are acetylated by CBP (K438, K443, K445, K533, K536 and K538 residues, on the bottom), important in its DNA binding; a NES sequence (amino acid sequences between 553 and 562, on the top) through which Nrf2 is exported from the nucleus by Crm1 protein; a K533 residue, important in SUMOylation process by Ubc9 protein; and S558 residue that is phosphorylated by AMPK, relevant in Nrf2 nuclear translocation. Neh2 is a degron domain. Keap1 and other ubiquitin ligases such as CRIF1 and WDR23 bind to Nrf2 trough this domain. ETGE and DLG motifs (on the top) are the sequences through which Keap1 binds to Nrf2. The DIDLID motif (on the top) is the region through WDR23 binds to Nrf2. Moreover, in this domain Nrf2 possess an α-helix with seven lysine amino acid residues (K44, K50, K52, K53, K56, K64 and K68, on the bottom), which are the ubiquitin amino acid acceptors; a NLS sequence (amino acid sequences between 42 and 53, on the bottom) important in its nuclear translocation through Karyopherin α1 and Karyopherin β1 importins; and S40 residue that is phosphorylated by PKCδ, important in Nrf2 nuclear translocation. Neh3 domain contains a second NLS sequence (amino acid sequences between 595 and 601, on the bottom); two Lys residues that are acetylated by CBP (K596 and K599, on the top), important in Nrf2 nuclear translocation; and K603, important lysine residue that is SUMOylated by Ubc9 protein. Through this domain, Nrf2 recruits CDH6 protein co-activator. Neh4 and Neh5 are transactivation domains. CBP and p300 as well as RAC3 bind to Nrf2 through this domain. Hrd1 an ubiquitin ligase, also binds to Nrf2 in this domain. A second NES sequence (amino acid sequences between 191 and 202, on the top) is localized in the Neh5 domain. Neh6 is a second degron domain. βTrCP ubiquitin ligase binds to Nrf2 through DSGIS motif (on the top), previous phosphorylation in S344 and S347 by GSK3β and DSAPGS motif (on the top). Finally, through Neh7 domain, Nrf2 interacts with RXRα protein, inducing Nrf2 repression. The figure construction was carried out following the next guideline "Guidelines for preparing color figures for everyone including the colorblind" [14].

2. Nrf2 protein

Nrf2 is a member of the Cap ´n´ Collar (CNC) transcription factors with a basic leucine zipper region (bZip). It has 605 amino acid residues, however the electrophoretic mobility indicates a 96–118 KDa molecular weight, caused by the abundance of acidic residues in its structure [4] and posttranslational modifications such as phosphorylation [5]. Nrf2 has 7 Neh domains (Neh1-Neh7) important in its activity and its repression [6]. Neh1 is implied in DNA binding and heterodimerization with small Maf proteins [6,7]. Neh2 and Neh6 are degron regions (an amino acid sequence or structure in a protein involved in its degradation), targeted by Keap1 and βTrCP, through ^{29}DLG^{31} and ^{79}ETGE^{82} motifs and ^{343}DSGIS^{347} and ^{382}DSAPGS^{387} motifs, respectively [8,9]. Neh3, Neh4 and Neh5 are transactivation domains through CHD6, CBP and RAC3 proteins association [10–12]. Finally, Neh7 domain is implicated in Nrf2 repression by RXRα protein [13] (Fig. 1).

Nrf2 is ubiquitously expressed [4] and regulates the expression of around 1055 genes [15], which have in its structure the cis-acting antioxidant response element (ARE, 5′-GTGACNNNGC-3′) [16]. ARE sequence is present in the promoter region of genes involved in the antioxidant and detoxifying response, cellular proliferation, metabolism, immune response, signaling, cell survival and cellular cycle. However, Nrf2 is considered the master regulator of the redox cellular state because its deletion or decrease in aging is associated with an increase in oxidative stress and cellular death [17,18].

3. Nrf2 regulation

Nrf2 is regulated at the transcriptional level by itself [19] and other transcription factors such as AhR [20], PPARγ [21], NF-κB [22], Sp-1, p53 [23], MEF2D [24], c-Jun, c-Myc [25] and BRCA1 [26]. Epigenetic mechanisms such as methylation of the Nrf2 promoter in CpG islands, H3 histone methylation and H4 histone acetylation are also involved in Nrf2 transcriptional regulation [27]. miRNAs such as miR-27a, miR-28, miR-34a, miR-93, miR129-5p, miR142-5p, miR-144, miR-153, miR-155, miR-200c, miR-340, miR-340-5p, miR-450a, miR-507 and miR-634 are able to regulate Nrf2 synthesis at the posttranscriptional level [28].

At the translational level, in homeostatic conditions, Nrf2 is regulated by mTORC1 in a cap-dependent process; however, in the presence of oxidant stimuli, such as H_2O_2 or α-lipoic acid, the regulation of Nrf2 is carried out in a cap-independent process by an Internal Ribosomal Entry Site (IRES) mechanism [29]. Finally, at the posttranslational level, Nrf2 is regulated by proteasomal degradation mainly by Keap1 dependent ubiquitination [30]; however, βTrCP [8], CRIF1 [31], Hrd1 [32] and WDR23 [33] are also able to induce Nrf2 ubiquitination and subsequent proteosomal degradation.

Keap1 is a Zn metalloprotein with 625 amino acid residues and 5 domains in its structure. The domains include the N-terminal region (NTR) [34], the Bric-a-brac, tramtrac, broad-complex/proxvirus zinc fingers (BTB/POZ) domain [6], implicated in Keap1 homodimerization and Cullin 3 (Cul3) association, the intervening region (IVR) [34], a linker between BTB and DGR domains, the Double glycine repeat/Kelch (DGR/Kelch) domain [6], important in Nrf2 repression and actin interaction, and the C-terminal region (CTR) [34]. Keap1 has some reactive cysteine residues, the C273, C288 and C297 in the BTB/POZ domain [35] and the C151 in the IVR domain [36], which are oxidized by Nrf2 inducers such as ROS, RNS, electrophilic compounds such as