ROS

RESEARCH HIGHLIGHTS

Nrf2 Silencing for Neuron Maturation

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ABSTRACT | Tightly regulated levels of reactive oxygen species (ROS) are instrumental in redox homeostasis and cell signal transduction. Nuclear factor E2-related factor 2 (Nrf2), a central regulator of cellular antioxidative and cytoprotective genes, plays a crucial role in controlling ROS levels and thereby oxidative stress injury. A recent study by Bell and associates reported in Nature Communications (2015 May 13; 6:7066. doi: 10.1038/ncomms8066) suggests that Nrf2 negatively impacts neuronal development in experimental models. This work along with others supports an essential role for ROS in normal development, and interruption of ROS signaling might negatively impact life and health.

KEYWORDS | Neuron maturation; Nrf2, Reactive oxygen species; Redox signaling

ABBREVIATIONS | Nrf2, nuclear factor E2-related factor 2; ROS, reactive oxygen species

CONTENTS

1. ROS: OXIDATIVE STRESS AND REDOX SIGNALING

For "free" utilization of molecular oxygen, aerobic organisms pay a price in the form of spending energy to produce antioxidant defenses to control the inevitable products of aerobic metabolism, i.e., reactive oxygen species (ROS), and counteract the detrimental effects of these species on cellular constituents, including nucleic acids, proteins, and lipids, among others. Failure of generating sufficient antioxidant defenses causes excessive accumulation of ROS, leading to oxidative stress and cell injury, contributing to aging and aging-related degenerative disorders, such as neurodegenerative disorders, cardiovascular diseases, and cancer. On the other hand,

regulated production of ROS is essential for phagocyte-mediated innate immunity [1]. Recent studies also reveal a central role for mitochondrial ROS in innate as well as cell-mediated immunity [2–4]. In addition, ROS, especially hydrogen peroxide, serve as second messengers to regulate cell signaling transduction [5, 6]. Indeed, redox signaling, like protein phosphorylation and dephosphorylation, is recognized as an important mechanism of cell signal transduction. Notably, an intimate crosstalk between cellular redox signaling and protein phosphorylation/dephosphorylation may operate coordinately to regulate cell physiology (**Figure 1**).

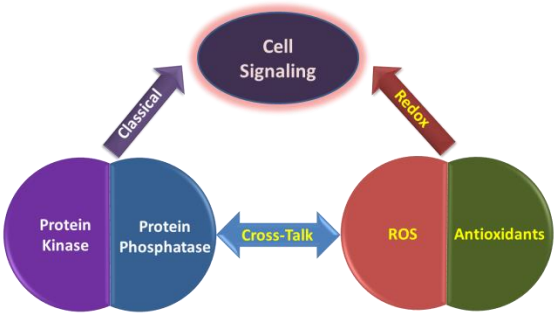

FIGURE 1. Schematic illustration of the molecular mechanisms of cellular redox signaling and protein phosphorylation/dephosphorylation and their cross-talk in cell signal transduction. In parallel with protein phosphorylation and dephosphorylation, redox reactions are also critically involved in normal cell signal transduction. Phosphorylation and dephosphorylation and redox signaling are also intertwined. One the one hand, kinase activation is involved in regulating redox status via affecting ROS formation and antioxidant gene expression. On the other hand, redox status also influences phosphorylation and dephosphorylation via inhibiting redox-sensitive protein phosphatases and/or activating redox-responsive protein kinases.

2. NRF2 SIGNALING AND ROS HOMEOSTASIS

Detoxification of ROS requires the coordinated action of a diverse of antioxidative enzymes/proteins including superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase, among many others. The transcription factor Nrf2 interacts with the antioxidant response element (ARE) to cause increased transcription of a number antioxidative and cytoprotective genes. Nrf2 is typically sequestered by its inhibitor Keap1 in the cytoplasmic compartment and dissociation/degradation of Keap1 leads to nuclear translocation of Nrf2 and the subsequent transactivation of a network of cytoprotective genes, with many involved in the metabolism of ROS (**Figure 2**). Indeed, deletion of Nrf2 gene causes decreased basal levels of cellular antioxidant defenses and sensitizes the animals to oxidative stress-associated pathophysiological conditions [7, 8].

3. NRF2 SIGNALING: POTENTIAL POSITIVE IMPACT ON NEURODEGENERATION

Knockout of Nrf2 gene increases the susceptibility to neurotoxin-induced neurodegeneration in both in vitro and in vivo models [9, 10]. Conversely, activation of Nrf2 signaling by chemoprotectants of diverse structures protects against neurodegeneration in experimental models [11]. Nrf2 signaling is also correlated with longevity in rodent models [12]. These experimental findings are in line with the current notion that oxidative stress plays a role in neurodegeneration and other aging-related conditions. In contrast to many studies on the detrimental effects of ROS, research on the physiological or beneficial role of ROS in the central nervous system is scarce.

4. NRF2 SIGNALING: POTENTIAL NEGATIVE IMPACT ON NEURON MATURATION

4.1. An Unknown Biological Rationale

Neurons consume large amounts of oxygen, but are equipped with relatively lower levels of antioxidant defenses as compared with astrocytes and cells in many other organs. The biological rationale behind this relative weak intrinsic antioxidant defenses in neurons is unknown. Oxidative environment in cells or subcellular compartments is known to affect cellular activities, such as insulin secretion by the pancreatic beta-cells [13]. It is possible that the relatively deficient antioxidant defenses may help maintain a

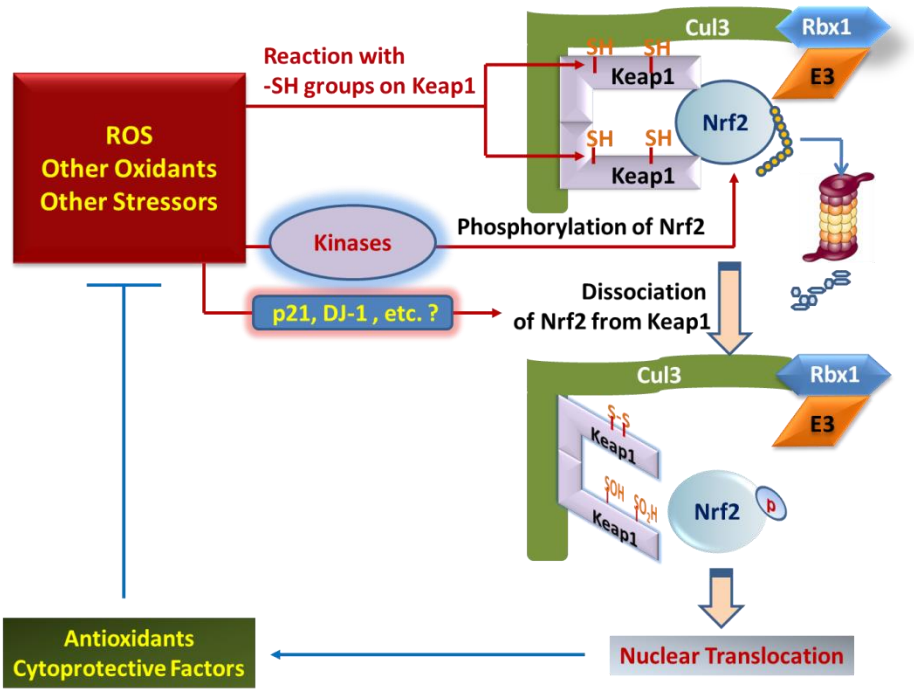

FIGURE 2. Schematic illustration of Nrf2 signaling and its role in controlling ROS and oxidative stress. ROS and other oxidants and stressors activate Nrf2 via diverse mechanisms, leading to degradation of Keap1 and the subsequent nuclear translocation of Nrf2 to cause transactivation of genes encoding antioxidants and other cytoprotective proteins. Such upregulated antioxidant defenses in turn promote metabolism and detoxification of ROS and other toxic species, contributing to protection against oxidative stress-associated tissue injury and disease processes.

specific cellular redox status that promotes neuronal development. Indeed, ROS are required for diverse physiological processes, including stem cell proliferation [14] and wound healing [6, 15].

4.2. An Important Study

In their work, Bell et al. using in vitro and in vivo models of neuronal development showed that Nrf2 signaling was repressed via an epigenetic mechanism during early development, and this epigenetic repression and the resulting redox status were required for neuron maturation [16]. The work first showed hypo-expression of Nrf2 in cortical neurons in both in vitro and in vivo and then demonstrated that the hypo-expression of Nrf2 in neurons was primarily due to hypo-acetylation/epigenetic repression of the Nrf2 promoter. By studying neurons at different stages of maturation, the authors showed that Nrf2 repression in neurons was developmentally regulated; neuronal Nrf2 expression became repressed during a developmental window early in neuronal commitment. This developmental silencing of Nrf2 expression was also associated with reduced expression of Nrf2-regulated antioxidative and cytoprotective genes [16].

To further investigate the potential negative involvement of Nrf2 signaling in neuronal development, the authors examined the impact of forced expression of Nrf2 during the developmental period in which it is ordinarily repressed. They demonstrated that forced expression of Nrf2 in neurons early in differentiation (around DIV4; DIV denotes days in vitro) at a time when Nrf2 was normally subject to repression was deleterious to neuronal development. The developmental abnormalities became apparent quickly (by DIV7), potentially because growth and

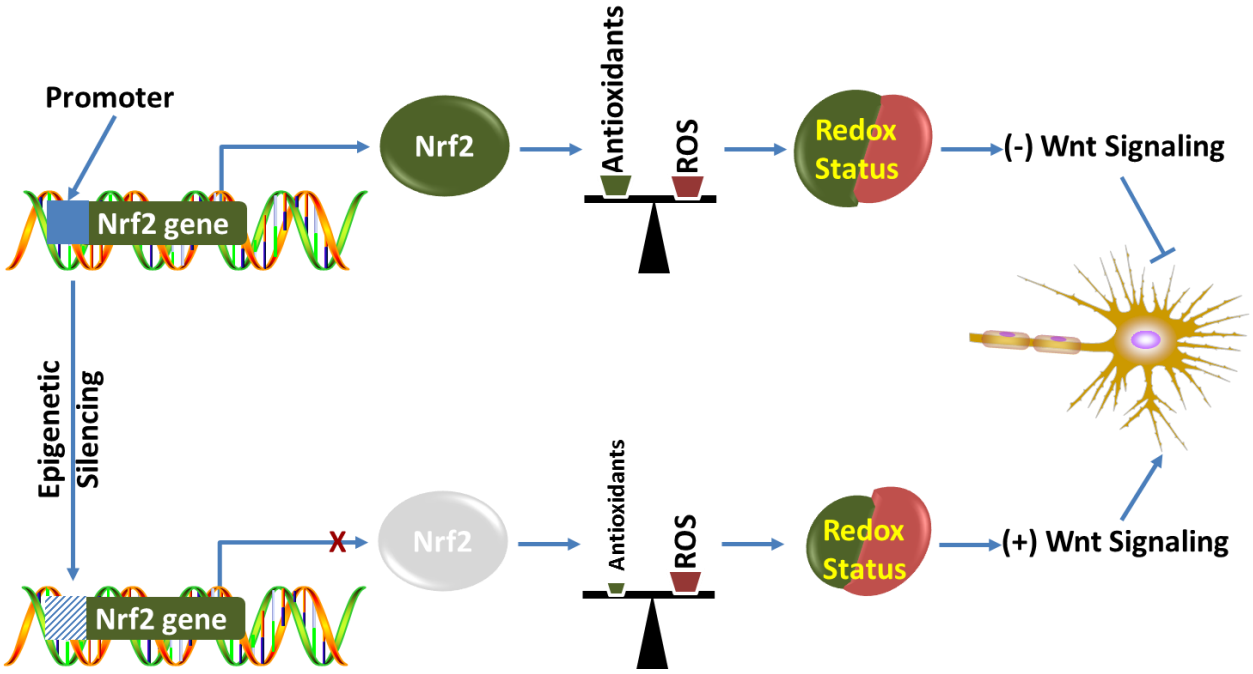

FIGURE 3. Schematic illustration of the role of epigenetic silencing of Nrf2 signaling in permitting neuron maturation. Nrf2 signaling activates antioxidant gene expression, which results in a redox status that would not be permissive for Wnt signaling, thereby leading to inhibition of neuron maturation. Epigenetic silencing of Nrf2 expression via hypo-acetylation and repression of the promoter activity of Nrf2 gene results in a redox status that is permissive for Wnt signaling that is critical for neuron maturation, including dendritic outgrowth and synaptogenesis.

electrophysiological maturation were rapidly occurring at this stage. Moreover, the developmental abnormalities were still observed later on (DIV17) as long as Nrf2 expression was initiated early, but not if expression was initiated later in development (day 14) [16]. These results led the authors to conclude that down regulation of Nrf2 signaling early in development might help establish a redox environment permissive for signaling pathways involved in neuron maturation during a critical developmental window over which particularly rapid electrical and morphological maturation took place [16]. Finally, the authors studied the potential negative impact of Nrf2 singling on key developmental pathways which are subjected to redox regulation. In this context, the redox-sensitive Wnt signaling pathway plays a critical part in neuronal differentiation, and both canonical (via β-catenin) and non-canonical (via Rac and JNK) Wnt signaling contribute to dendritic development and synaptogenesis, characteristic features of neuron maturation. The authors showed that the Wnt signaling pathway was repressed by Nrf2 signaling, and importantly, the deleterious effects of Nrf2 expression on dendritic outgrowth and arborization could be rescued by artificially activating canonical and non-canonical Wnt signaling or JNK [16].

Collectively, the large array of data reported in this elegantly carried out study support a model whereby Nrf2-driven antioxidant defenses repress the activity of redox-sensitive Wnt and JNK pathways, leading to deficits in neuron maturation (**Figure 3**). This notion offers a biological reason behind the developmental epigenetic silencing of Nrf2 expression, that is, by down regulation of Nrf2-dependent cellular antioxidant defenses, a more permissive redox environment may be provided for supporting redox-

sensitive signal transduction pathways critical for neuron maturation [16].

4.3. Important Implications

The work by Bell et al. has important mechanistic and practical implications. Mechanistically, this is the first comprehensive study that shows a potential for Nrf2 to negatively impact neuronal development and thereby offers a biological reason for epigenetic silencing of Nrf2 signaling during neuron maturation. Practically, this work suggests a possibility for over-supplementation of antioxidants (such as during pregnancy or early development) to negatively impact neuronal development. In this context, future investigation of the effects of antioxidant supplementation on neuronal development in both animal models and humans is warranted.

This elegant study by Bell and coworkers adds another excellent example to the growing list of cases that support an essential role for ROS in life and health. In this context, formation of ROS by aerobic metabolism in mammals should not be considered solely as an evolutional "defect", but rather also a regulatory mechanism for maintaining normal physiological homeostasis.

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