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Commentary

The Nrf2 cell defence pathway: Keap1-dependent and -independent mechanisms of regulation

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ABSTRACT

The transcription factor Nrf2 (NF-E2-related factor 2) plays a vital role in maintaining cellular homeostasis, especially upon the exposure of cells to chemical or oxidative stress, through its ability to regulate the basal and inducible expression of a multitude of antioxidant proteins, detoxification enzymes and xenobiotic transporters. In addition, Nrf2 contributes to diverse cellular functions including differentiation, proliferation, inflammation and lipid synthesis and there is an increasing association of aberrant expression and/or function of Nrf2 with pathologies including cancer, neurodegeneration and cardiovascular disease. The activity of Nrf2 is primarily regulated via its interaction with Keap1 (Kelch-like ECH-associated protein 1), which directs the transcription factor for proteasomal degradation. Although it is generally accepted that modification (e.g. chemical adduction, oxidation, nitrosylation or glutathionylation) of one or more critical cysteine residues in Keap1 represents a likely chemico-biological trigger for the activation of Nrf2, unequivocal evidence for such a phenomenon remains elusive. An increasing body of literature has revealed alternative mechanisms of Nrf2 regulation, including phosphorylation of Nrf2 by various protein kinases (PKC, PI3K/Akt, GSK-3β, JNK), interaction with other protein partners (p21, caveolin-1) and epigenetic factors (micro-RNAs -144, -28 and -200a, and promoter methylation). These and other processes are potentially important determinants of Nrf2 activity, and therefore may contribute to the maintenance of cellular homeostasis. Here, we dissect evidence supporting these Keap1-dependent and -independent mechanisms of Nrf2 regulation. Furthermore, we highlight key knowledge gaps in this important field of biology, and suggest how these may be addressed experimentally.

1. Introduction

The exposure of cells to a range of environmental toxicants, mutagens and potential carcinogens has been linked to the pathogenesis of a broad range of diseases including cancer, neurodegenerative disease, cardiovascular disease and inflammation [1]. To protect against such insults, eukaryotic cells have developed complex signalling cascades to detoxify potentially harmful substances and maintain cellular redox homeostasis. One of these signalling cascades is responsible for the induction of cytoprotective and detoxifying enzymes consisting of phase I (cytochrome P450s) and phase II (detoxifying and antioxidant proteins) enzymes [2]. The co-ordinated expression of these genes removes the insult and attempts to restore the cell to a basal state by conferring a resistance to stress, thus preventing damage to cellular components sensitive to redox changes (i.e. proteins, lipids and DNA) [3]. The ubiquitously expressed cap'n'collar bZip transcription factor Nrf2 is largely responsible for the basal and inducible expression of proteins involved in drug metabolism, the oxidative stress response and cytoprotection. Supplementary to its primary role in cytoprotection, Nrf2 is also linked to differentiation, proliferation, growth, apoptosis and it is thought that Nrf2 has evolved from an original role in haematopoiesis and the regulation of cell differentiation from early lineages [4]. Whilst a study by Chan et al., showed that Nrf2 is not essential for growth,

Abbreviations: DEA-NO/AM, acetoxymethylated diethylamine-NONO-ate; LC-ESI MS/MS, liquid chromatography electronspray ionisation tandem mass spectrometry; MRM, multiple reaction monitoring; tBHQ, tert-butylhydroquinone; CDDO-Me, methyl-2-cyano-3,12 dioxoolean-1,9 diene-28-oate; CDDO-Im, 1[2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl] imidazole.

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development or erythropoiesis in mammalian cells, these authors suggest that Nrf2 could still have originally played this role in an avian system, due to observations made by Itoh et al. of high Nrf2 expression in chicken hematopoietic cells [5,6]. Furthermore, there is still evidence of Nrf2s role in haematopoiesis in a mammalian system as it regulates the expression of haemoxygenase-1 (HO-1) which is involved in the handling of iron [7]. In addition, recent findings show that Nrf2 is functionally involved in lipid deposition in the liver. Using proteomic analysis (iTRAQ) to compare wild type (WT) and Nrf2 knockout ($Nrf2^{-/-}$) mice, it was shown that basally, Nrf2 regulates a number of proteins involved in the synthesis and metabolism of fatty acids and other lipids [8]. The most potent known Nrf2 inducers; the triterpenoids CDDO-Me and CDDO-Im (synthesised from oleanolic acid) are lipid soluble molecules which have been shown to reduce the accumulation of lipids in the livers of mice on a high fat diet via the Keap1/Nrf2 pathway [9]. The importance of the basal control of lipid metabolism by Nrf2 is not understood. However it is possible that this process is under the control of Nrf2 when the cell is in a basal, energy-sufficient state yet, in times of stress and when Nrf2 is induced, lipid synthesis and other biosynthetic pathways are down-regulated to compensate for the energy requirements of cell defence mechanisms.

A vital factor in the functioning of many transcription factors is their spatio-temporal regulation and this is no different in the case of Nrf2; it is just as important that Nrf2 is switched on in response to a stimulus as it is that it is switched off when the stimulus has been removed. It is for this reason that this pathway is highly regulated with a number of different mechanisms responsible for preventing the aberrant activation of Nrf2. One of the most important mechanisms that regulate the cells response to inflammatory, hypoxic, oxidative and xenobiotic stimuli is proteasomal degradation; and the Nrf2 pathway is no exception to this. [10]. In unstressed conditions, the level of Nrf2 protein in the cell is maintained at very low levels by its inhibitor Keap1, which sequesters Nrf2 in the cytosol and facilitates its degradation via the proteasome. Under conditions of stress or in the presence of Nrf2 activating compounds, this degradation is hindered and Nrf2 translocates to the nucleus. Here, Nrf2 heterodimerises with small masculoaponeurotic fibrosarcoma (Maf) proteins which in turn facilitate the binding of Nrf2 to the Antioxidant Response Element (ARE), a *cis*-acting enhancer sequence (TCAG/CXXXGC) in the promoter region of Nrf2-regulated genes [11,12] (Fig. 1a). These Nrf2-regulated genes can be classified into phase II xenobiotic-metabolizing enzymes antioxidants, molecular chaperones, DNA repair enzymes, and anti-inflammatory response proteins [13] and they reduce reactive compounds such as electrophiles and free radicals to less toxic intermediates whilst increasing the ability of the cell to repair any damage ensued. Importantly, Nrf2 has been shown to possess an ARE sequence within its own promoter region providing a platform for Nrf2 to initiate its own transcription further enhancing the adaptive cell defence response [11]. Following its nuclear import, Nrf2 recruits transcriptional machinery to effectively transactivate the ARE-driven genes. This machinery includes co-activators such as receptor associated co-activator (RAC3) which initiates the transactivation domain of Nrf2 whilst the presence of other co-regulators such as CREB binding

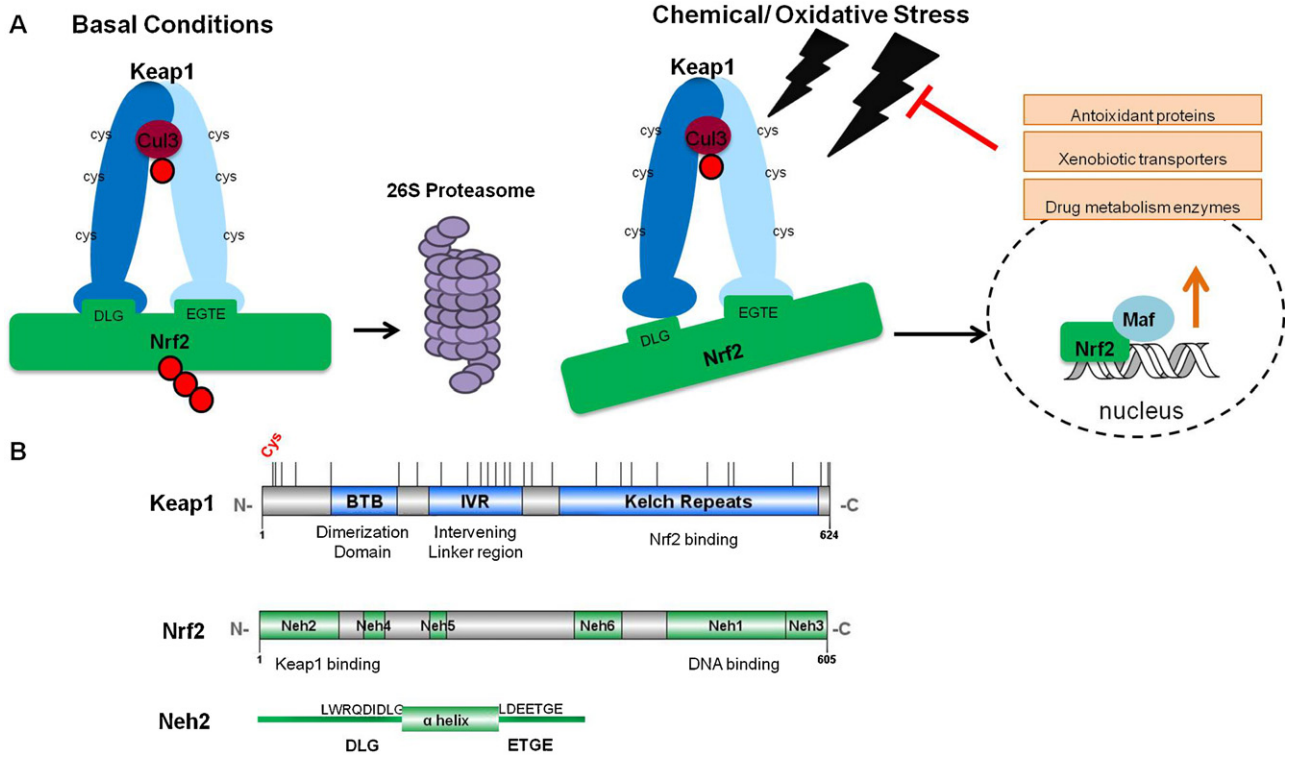

Fig. 1. (A) Schematic overview of the Nrf2 pathway. Under basal conditions, Nrf2 is sequestered in the cytosol by a Keap1 homodimer which facilitates the ubiquitination and proteasomal degradation of Nrf2. When the cell is faced with an insult such as chemical or oxidative stress, a conformational change in Keap1 mediated via its reactive cysteine residues results in the release of Nrf2 from one Keap1 molecule. Nrf2 can no longer be ubiquitinated and degraded therefore Keap1 becomes fully saturated with Nrf2, allowing newly synthesised Nrf2 to accumulate and translocate to the nucleus. Here Nrf2 heterodimerises with small Maf proteins and binds to the antioxidant response element (ARE). This activates the expression of a battery of genes responsible for removing the insult, conferring increased resistance to stress and returning the cell to a basal state. (B) Keap1 and Nrf2 Protein Domains. Keap1 contains a number of functional domains including the Broad complex, Tramtrack and Bric-a-brac (BTB), the intervening linker domain (IVR), the double glycine/Kelch repeats and the C-terminal region. The BTB is responsible for the dimerisation of two Keap1 molecules whilst the Kelch repeats contain the region responsible for binding Nrf2, facilitated in particular by a number of arginine residues (Arg-380, -415, -483). Keap1 contains a number of reactive cysteine residues also highlighted. Nrf2 contains Neh1-6 domains of which Neh1 binds to the ARE within DNA whilst Neh4/5 are transactivation domains. Neh2 is responsible for binding to Keap1 via the ^{29}DLG^{31} and ^{79}ETGE^{82} motifs which flank an α helix region containing the lysine residues for Keap1-mediated ubiquitination.

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