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Review

Nrf2: Molecular and epigenetic regulation during aging

Silva-Palacios A.[a,b,c], Ostolga-Chavarría M.[b], Zazueta C.[b], Königsberg M.[a,*]

[a] Departamento de Ciencias de la Salud, DCBS, Universidad Autónoma Metropolitana-Iztapalapa, Ciudad de Mexico, 09340, Mexico
[b] Departamento de Biomedicina Cardiovascular, Instituto Nacional de Cardiología-Ignacio Chávez, Ciudad de Mexico, Mexico
[c] Posgrado en Biología Experimental, UAM-I, Mexico

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ABSTRACT

Increase in life-span is commonly related with age-related diseases and with gradual loss of genomic, proteomic and metabolic integrity. Nrf2 (Nuclear factor-erythroid 2-p45 derived factor 2) controls the expression of genes whose products include antioxidant proteins, detoxifying enzymes, drug transporters and numerous cytoprotective proteins. Several experimental approaches have evaluated the potential regulation of the transcription factor Nrf2 to enhance the expression of genes that contend against accumulative oxidative stress and promote healthy aging. Negative regulators of Nrf2 that act preventing it´s binding to DNA-responsive elements, have been identified in young and adult animal models. However, it is not clearly established if Nrf2 decreased activity in several models of aging results from disruption of that regulation. In this review, we present a compilation of evidences showing that changes in the levels or activity of Keap1 (Kelch-like ECH associated protein 1), GSK-3β (glycogen synthase kinase-3), Bach1, p53, Hrd1 (E3 ubiquitin ligase) and miRNAs might impact on Nrf2 activity during elderly. We conclude that understanding Nrf2 regulatory mechanisms is essential to develop a rational strategy to prevent the loss of cellular protection response during aging.

1. Introduction

The need to understand why we become old and the desire to prevent it, have accompanied humanity since ancient times. This question has taken relevance in the last decades because the elderly population has dramatically augmented on a global scale (Horn, 2015). A special case of successful aging in humans are the centenarians who, by definition, are extremely old people that show all the signs and characteristics of a prolonged aging process, while avoiding or postponing the beginning of the decline related to age (Franceschi and Bonafè, 2003; Ismail et al., 2016; Kheirbek et al., 2017). Therefore, the study of centenarians has shed light on some basic mechanisms of aging and longevity in humans, allowing us to consider other variables-difficult to study in animal models-such as demography, population genetics and variables related to cultural habits, such as lifestyle and personality, typical of humans and that undoubtedly play an important role in aging and longevity (Franceschi and Garagnani, 2016; Franceschi et al., 2017a). Molecular studies focused on elucidating the mechanisms that safeguard aging in humans have been addressed. For example, Lattanzi et al. (2014) showed that rapamycin affects pre-lamin A levels and favors the recruitment of nuclear protein 53BP1, thus mimicking the nuclear environment observed in cells of centenarian individuals (95–105 years). Still, more experiments must be done in order to understand their successful aging.

Although medical research and basic science have managed to expand longevity, both in laboratory models and in humans, lifespan increase does not always go hand in hand with health span. This is clearly seen in a large part of the human population, where there is an important period of frailty during the last years of life (Herndon et al., 2002). Older adults have a greater susceptibility to develop pathological conditions, which augment the number of hospitalizations, generating a financial deficit in public health systems. Decreased health and fitness, together with greater difficulty to overcome illnesses or physical stress, lead to permanent loss of functions and increased mortality in the elderly (Lewis et al., 2010; Camici et al., 2015). Although it is believed that these traits in old age are due to a gradual loss of genomic, proteomic and metabolic integrity (Lewis et al., 2010), the accurate mechanisms that induce this deterioration remain unknown. The identification of those pathways would undoubtedly bring opportunities to improve quality of life during aging.

In aging models such as yeast, worms, and flies, lifespan has increased from 40 to more than 200% by mutating genes such as *age-1/PI3-K* (phosphoinositide 3-kinase) (Friedman and Johnson, 1988) or *daf-2/IGF-1* (insulin like growth factor-1) (Kenyon et al., 1993).

* Corresponding authorat: Departamento de Ciencias de la Salud, División de Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana-Iztapalapa, A.P. 55-525, C.P 09340, Ciudad de Mexico, Mexico.
E-mail addresses: mkf@xanum.uam.mx, mina.konigsberg@fulbrighmail.org (M. Königsberg).

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However, until recently, not much attention was paid to the enhanced health span in these models. The results in this respect are ambiguous; Tissenbaum and co-workers (Bansal et al., 2015) reported that *Caenorhabditis elegans* long-lived mutants tend to have a longer period of frailty. In contrast, the Kenyon´s group found that some *daf-2* mutants have extended movement ability, representing a better health state (Podshivalova et al., 2017)

Hence, it is still not clear if the aging process is ruled by events underlying both, health and longevity, or if the events that regulate increased longevity are independent of those who control health span, thus resulting in an extension of frailty (Newell Stamper et al., 2018). Therefore, finding and understanding the pathways driving lifespan extension and healthy aging is the major challenge to be resolved by geroscience (Kennedy et al., 2014; Kohanski et al., 2016).

Harman´s Free Radicals Theory of Aging was one of the most prominent theories for a long time (Harman, 1956). However, in recent years, many researchers have questioned its validity, as experimental data are incompatible with its basic premises (Buffenstein et al., 2008; Pérez et al., 2009a,b). Data obtained from transgenic manipulations of the antioxidant system have shown that life expectancy in those animals is not related neither with the levels of antioxidant enzymes nor with oxidative damage accumulation, indicating that it is necessary to modify the hypothesis proposed by Harman.

However, increased oxidative stress/damage correlate with several diseases; thus, it seems plausible that healthy aging, if not longevity, might be linked to oxidative stress resistance (Done and Traustadóttir, 2016). Moreover, the way in which the cells respond to changes in the environment, nutrition, lifestyle (i.e. what has been called the *exposome)*, defines which genes are to be expressed in order to generate the metabolome; and it has been proposed that these epigenetic changes are regulated by the variations in the redox state (Jones, 2015). So, the molecules that sense these redox variations and that activate the signaling pathways in response to them have generated great interest in the aging field. One of these molecules is the transcription factor Nrf2 (Nuclear factor-erythroid 2-p45 derived factor 2), and its regulation and downstream pathways have received special attention.

It has been shown that animals with deficient Nrf2 have an increased prevalence of age related diseases such as heart disease (Shanmugam et al., 2017), skeletal muscle loss (Ahn et al., 2018) cancer (Volonte et al., 2013), atherosclerosis and liver damage (Collins et al., 2012; Duarte et al., 2017), visual deficit (Larabee et al., 2016; Nakagami, 2016), glomerulonephritis (Yoh et al., 2001), together with shortened life expectancy and premature senescence (Yoh et al., 2001; Volonte et al., 2013). In this sense, Strong et al. (2016) evaluated the competence of several agents to increase healthy lifespan and reported that some molecules such as Protandim (a mixture of botanical extracts that activate Nrf2), extended lifespan. Although Nrf2 activation has mostly been associated with beneficial and cytoprotective effects, its overexpression has also been related with deleterious outcomes. For example, constitutive Nrf2 activation in cancer cells can provide them with antioxidant protection and chemotherapeutic drug resistant (Hybertson and Gao, 2014). Therefore, Nrf2 activation or overexpression might not be enough to prolong healthy life in aging models, thus demanding to study the molecular and epigenetic regulation of this transcription factor as a central part in the search for mechanisms that might help promote healthy aging and increased life expectancy.

2. Nrf2 and its role in aging

The transcription factor Nrf2 emerged from obscurity in 1997 at the University of Tsukuba by biochemist Masayuki Yamamoto (Garber, 2012). It regulates approximately 250 genes involved in cellular homeostasis, including antioxidant proteins, detoxifying enzymes, drug transporters and numerous cytoprotective proteins (O'Connell and Hayes, 2015).

Nrf2 heterodimerization with small Maf proteins (MafG, MafK, MafF) is required for efficient binding to the ARE/EpRE (antioxidant response element/electrophilic response element), that mediates the transcription of Nrf2-regulated genes such as NAD(P)H:quinone-oxidoreductase-1 (NQO1), γ-glutamyl cysteinyl synthetase (γ-GCS), heme-oxygenase-1 (HO-1) and others.

In normal conditions, Nrf2 is associated with Keap1 (Kelch-like ECH-associated protein 1) a multi-domain protein rich in cysteines that is linked with actin filaments (Kang et al., 2004), together with Cullin-3 (Cul-3) and Rbx1 (RING-box protein 1) proteins, forming a complex of ubiquitin-ligase E3 that allows its constant degradation through the proteasome, in a process mediated by ubiquitin (Su et al., 2015). The covalent conjugation of proteins by ubiquitin, involves three enzymatic processes: activation (E1), conjugation (E2) and ligation (E3). Nrf2 serves as a substrate and Cul-3 functions as a scaffolding protein forming the ligase E3 complex with the Rbx1 protein, which recruits the E2 enzyme. Keap1 binds to Cul-3 and to the substrate leading to Nrf2 ubiquitination and proteasomal degradation. Exposure to reactive oxidants or electrophiles leads to the oxidation of critical cysteine residues within Keap1 (Cys^{151}, Cys^{273}, Cys^{288}). Such cysteine residues once oxidized (or chemically modified), release Nrf2 and prevent its ubiquitination; increasing its half-life from 15 to 180 min (Li et al., 2005; Lewis et al., 2010; Niture et al., 2010).

The time that Nrf2 remains active depends on the stimulus generated by the inductor, but also on the age of the animals. In line with this, our group observed that tert-butylhydroquinone (tBHQ) promotes Nrf2 binding to ARE only up to 30 min in primary astrocytes obtained from old rats in comparison with the astrocytes isolated from young rats, that maintain this response up to 180 min (Alarcón-Aguilar, et al., 2014).

Senile animals and older adults (> 65 years) have lower nuclear content and diminished Nrf2 activation than younger individuals (Safdar et al., 2010; Gounder et al., 2012). Nrf2 protein and mRNA expression declines with age in several tissues such as brain (Duan et al., 2009) and heart (Ungvari et al., 2011); these has been related to a decrease in the Nrf2 target genes NQO1, γ-GCS, HO-1, along with an increase in several NF-κB (Nuclear factor kappa B) target genes, such as ICAM-1 (intercellular adhesion molecule 1) and IL-6 (interleukin-6) (Ungvari et al., 2011). It has been reported that Nrf2 increased activity promotes healthy aging in mammals (Lewis et al., 2015), however, classic inductors of this pathway have had variable outcomes in different aging models. For example, tBHQ induces Nrf2 activity in primary cultured astrocytes from old rats (Alarcón-Aguilar et al., 2014), but has no effect on heart tissue from aged rats (Silva-Palacios et al., 2017), unraveling the complexity of Nrf2 regulation.

SKN-1, the orthologous of mammalian Nrf2 in *C. elegans*, is considered a relevant aging modulator of this nematode (Blackwell et al., 2015) and its downregulation compromises the organism's lifespan (Bishop and Guarente, 2007). Also, the activation of SKN-1 dependent signaling pathways preserves proteostasis network and/or confers resistance to oxidative stress, preventing aging associated damages in *Drosophila melanogaster* and *C. elegans* (Tsakiri et al., 2013; Ewald et al., 2015). These results support the premise that Nrf2 activates conserved mechanisms that promote healthy aging. However, it cannot be discarded that Nrf2 activity is also an essential regulator of longevity. Buffenstein and co-workers (2015) studied 10 different rodent species, representing five phylogenetic distinct families, and found an association between Nrf2 activity and maximum lifespan potential (MLSP), suggesting that as species independently evolved longer lifespans, they also augmented Nrf2 activity (Lewis et al., 2015). However, in contrast to Nrf2 activity, total Nrf2 protein levels did not show a significant relationship with MLSP. The important result in this case was the decrease of Keap1 levels, which allowed Nrf2-ubiquitination evasion and degradation. Moreover, they also proposed two other Nrf2 regulators, p62/SQSTM1 (a protein related with autophagy) and β-TrCP (an E3 ubiquitin ligase) promoting its proteasomal degradation, (both will be discussed later) (Rada et al., 2011; Lewis et al., 2015).