Redox Biology 13 (2017) 444–451

Contents lists available at ScienceDirect

Redox Biology

journal homepage: www.elsevier.com/locate/redox

Research Paper

NRF2 deficiency replicates transcriptomic changes in Alzheimer's patients and worsens APP and TAU pathology

Ana I. Rojo[a,*], Marta Pajares[a], Patricia Rada[b], Angel Nuñez[c], Alejo J. Nevado-Holgado[f], Richard Killik[d], Fred Van Leuven[e], Elena Ribe[f], Simon Lovestone[f], Masayuki Yamamoto[h], Antonio Cuadrado[a,g,**]

[a] Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), ISCIII. Instituto de Investigaciones Biomédicas "Alberto Sols" UAM-CSIC. Instituto de Investigación Sanitaria La Paz (IdiPaz), and Department of Biochemistry, Faculty of Medicine, Autonomous University of Madrid, Madrid, Spain
[b] Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), ISCIII, Madrid, Spain. Instituto de Investigaciones Biomédicas "Alberto Sols" UAM-CSIC. Instituto de Investigación Sanitaria La Paz (IdiPaz); and Department of Biochemistry, Faculty of Medicine, Autonomous University of Madrid, Madrid, Spain
[c] Department of Anatomy, Histology and Neuroscience, Autonomous University of Madrid, Madrid, Spain
[d] King's College London, Institute of Psychiatry, Psychology and Neuroscience, The Maurice Wohl Clinical Neuroscience Institute, Camberwell, London, UK
[e] Experimental Genetics Group-LEGTEGG, Department of Human Genetics, KU Leuven, Leuven, Belgium
[f] Department of Psychiatry, Warneford Hospital, University of Oxford, OX3 7JX UK
[g] Cellular and Molecular Medicine Department, Radiobiology Laboratory, "Victor Babes" National Institute of Pathology, Bucharest, Romania
[h] Department of Medical Biochemistry, Tohoku University Graduate School of Medicine, 2-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan

ARTICLE INFO

Keywords:
NRF2
Alzheimer's disease
Transcriptomics
Proteotoxicity
Neuroinflammation
Oxidative stress

ABSTRACT

Failure to translate successful neuroprotective preclinical data to a clinical setting in Alzheimer's disease (AD) indicates that amyloidopathy and tauopathy alone provide an incomplete view of disease. We have tested here the relevance of additional homeostatic deviations that result from loss of activity of transcription factor NRF2, a crucial regulator of multiple stress responses whose activity declines with ageing. A transcriptomic analysis demonstrated that NRF2-KO mouse brains reproduce 7 and 10 of the most dysregulated pathways of human ageing and AD brains, respectively. Then, we generated a mouse that combines amyloidopathy and tauopathy with either wild type (AT-NRF2-WT) or NRF2-deficiency (AT-NRF2-KO). AT-NRF2-KO brains presented increased markers of oxidative stress and neuroinflammation as well as higher levels of insoluble phosphorylated-TAU and Aβ*56 compared to AT-NRF2-WT mice. Young adult AT-NRF2-KO mice exhibited deficits in spatial learning and memory and reduced long term potentiation in the perforant pathway. This study demonstrates the relevance of normal homeostatic responses that decline with ageing, such as NRF2 activity, in the protection against proteotoxic, inflammatory and oxidative stress and provide a new strategy to fight AD.

1. Introduction

In spite of the efforts made over the last decades, the etiology of Alzheimer's disease (AD) remains largely unknown. This is in part caused by incomplete reproduction of the human pathology in animal models merely exhibiting the proteinopathy associated to deposition of amyloid-beta (Aβ) and hyperphosphorylation of TAU. Current AD-models do not reflect molecular mechanisms that correlate with the general decline of homeostatic capacity during ageing and may contribute significantly to the disease process. These mechanisms include oxidative, inflammatory and metabolic stress, which may precede the proteinopathy in prodromal and early phases of sporadic AD [1].

In this study, we have addressed this problem in the context of deficiency in the Nuclear factor (erythroid-derived 2)-like 2 (NRF2), the master regulator of homeostatic responses [2–6]. The NRF2 transcriptional signature provides an armamentarium to adapt reactive oxygen species signaling, inflammation and metabolism to normal physiological needs [7,8]. Its transcriptional acivity declines with ageing [9,10]. Also, in Hutchinson-Gilford progeria syndrome, characterized by premature ageing, NRF2 is mislocated in the nuclear periphery, resulting in impaired NRF2 activity and consequently increased chronic oxidative stress while NRF2-activators restore viability in an animal model of this

* Corresponding author.
** Corresponding author at: Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), ISCIII. Instituto de Investigaciones Biomédicas "Alberto Sols" UAM-CSIC. Instituto de Investigación Sanitaria La Paz (IdiPaz), and Department of Biochemistry, Faculty of Medicine, Autonomous University of Madrid, Madrid, Spain.
E-mail addresses: airojo@iib.uam.es (A.I. Rojo), antonio.cuadrado@uam.es (A. Cuadrado).

http://dx.doi.org/10.1016/j.redox.2017.07.006
Received 31 May 2017; Received in revised form 28 June 2017; Accepted 3 July 2017
Available online 05 July 2017

Table 1
Microarray validation by qRT-PCR. The expression data obtained using microarrays for selected genes was validated using qRT-PCR analysis. Fold of change in mRNA of the indicated genes was calculated relative to NRF2-WT brains and normalized to the average expression of *ActB, Tbp* and *Gapdh* house keeping genes. Data are mean ± SEM (n = 4).

		Microarray		qRT-PCR		
Gene Entrez ID	Gene name	Effect change	p-value (FDR/BH)	NRF2-WT	NRF2-KO	p-value (t-student)
14862	*Gstm1*	−3,87	0.0082	1.00 ± 0.11	0.23 ± 0.08	0.001
16653	*Kras*	−5.10	0.0022	1.00 ± 0.13	0.64 ± 0.07	0.042
69368	*Wdfy1*	−6.97	0.00043	1.00 ± 0.12	0.60 ± 0.09	0.023
114565	*Zbtb21*	11.36	0.000078	1.00 ± 0.44	4.00 ± 0.40	0.002

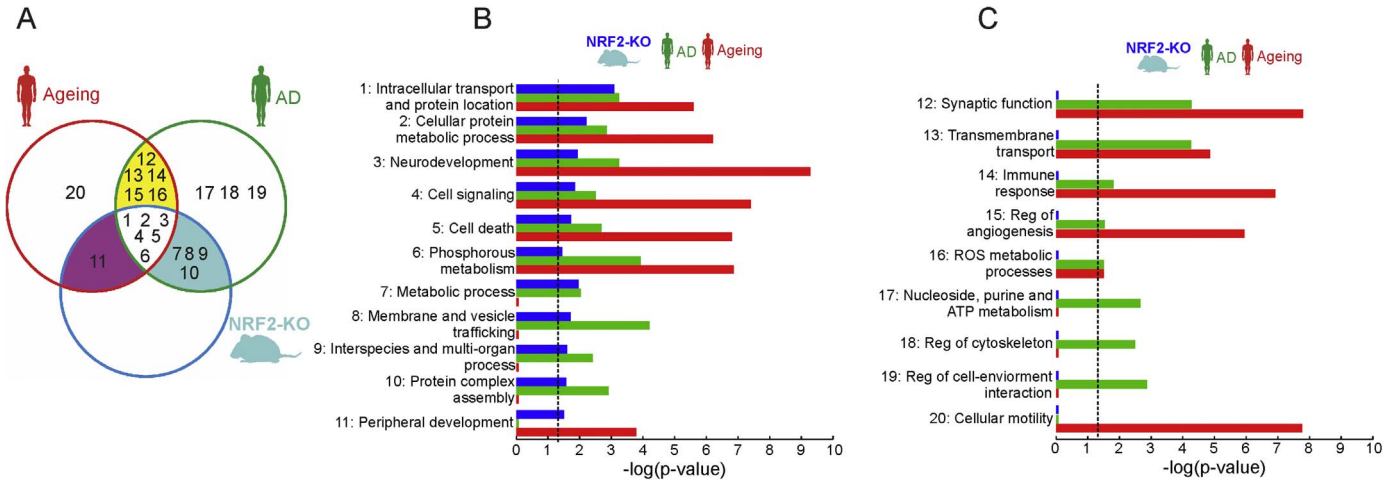

Fig. 1. *Comparison of the functional pathways altered in brain of NRF2-KO mice vs. human ageing and AD cohorts.* Altered gene expression in NRF2-KO mice was analyzed in brain samples from 11-months-old NRF2-WT (n = 4 males) vs. NRF2-KO mice (n = 4 males). Comparison with human ageing and AD brain cohorts was done as indicated in Material and Methods. A, Venn diagram showing the overlapping distribution of functional pathways in AD, ageing and NRF2-null brains. Cluster numbers correspond to: 1, regulation of cell signaling; 2, phosphorous and phosphate metabolism; 3, cellular protein metabolic process; 4, neurodevelopment; 5, regulation of cell death; 6, protein complex assembly; 7, intracellular transport and protein location; 8, nucleoside, purine and ATP metabolism; 9, regulation of cell cycle; 10, acetylcholine metabolism; 11, transmembrane transport of ions; 12, synaptic function; 13, cognition, learning and memory; 14, host response to infection; 15, lipid and alcohol metabolism; 16, interspecies and multi-organ modulation; 17, secretory and exocytic pathways; 18, regulation of membrane and vesicles; 19, regulation of cytoskeleton; 20, regulation of angiogenesis; 21; protein modification by conjugation; 22, regulation of cell-environment interaction; 23, glucose metabolism; 24, cell morphogenesis; 25, immune response; 26, ROS metabolic process; 27, cellular motility; 28, regulation of transmembrane transport; 29, development of peripheral tissues. B and C, summary of selected categories as calculated by DAVID. The categories are ranked according to -$\log_{10}$ P-values.

syndrome [11]. Moreover, male mice pharmacologically treated with a NRF2-activator exhibit longer lifespan [12].

Ageing is the main risk factor for AD and some scattered studies have noted the correlation between deficits in NRF2 and AD. In humans, one haplotype allele in the *NFE2L2* gene promoter, coding NRF2, was associated with an earlier onset of AD, implying that common variants of the *NFE2L2* gene might affect AD progression [13]. In animal models, the relevance of NRF2 has been analyzed in transgenic mice with amyloidopathy [14,15] or tauopathy [16,17], but not combined, and the main focus was on the role of NRF2 in proteostasis.

Here, we have performed a transcriptomics study comparing pathways altered in elderly and AD brains with those of NRF2-knockout mouse brains. From this reverse translational approach, we have further combined expression of the human APP^{V717I} and TAU^{P301L} transgenes [18] with lack of NRF2 expression. The absence of NRF2 led to earlier onset of the disease, with more severe amyloidopathy and tauopathy and exacerbated cognitive defects. This study demonstrates for the first time the importance of homeostatic functions regulated by NRF2 in the brain pathophysiology of AD and represents a new tool for interventional studies.

2. Material and methods

2.1. Transgenic mice

Colonies of NRF2-KO mice and NRF2-WT littermates were described previously [19]. Heterozygous APP^{V717I} mice (FVB/N), expressing the $hAPP_{695}$ isoform with the V717I mutation under the control of the mouse *Thy1* gene promoter, were crossed with C57/BL6j-NRF2-WT (APP-NRF2-WT) or C57/BL6j-NRF2-KO (APP-NRF2-KO). Similarly, TAU^{P301L} mice (FVB/N), expressing homozygously the longest isoform of protein TAU with the P301L mutation (TAU 4 R/2 N P301L) under control of the same mouse *Thy1* gene promoter, were crossed with C57/BL6j-NRF2-WT (TAU-NRF2-WT) or C57/BL6j-NRF2-KO (TAU-NRF2-KO). APP/TAU-NRF2-WT (AT-NRF2-WT) and APP/TAU-NRF2-KO (AT-NRF2-KO) in C57/BL6j background were obtained by crossing the above described genotypes for more than eight generations. Genotypic characterization of the APP^{V717I} and TAU^{P301L} transgenic mice was described previously [18,20,21].

2.2. RNA microarray expression analysis

Total mouse RNA was obtained from brain tissue by Trizol extraction (Invitrogen, California, USA) according to manufacturer's instruction and as previously described [22]. RNA quality was determined using a bioanalyzer (Agilent, California, USA). RIN values of all samples were greater than 9.5. For expression analysis 50 ng of each total RNA sample was converted and amplified to cDNA using the Ovation® Pico WTA System V2 according to manufacturer's instructions. Products were then hybridized on Mouse Gene 2.0 ST arrays containing probe sets for > 28,000 coding and > 7000 non-coding transcripts. The functional clustering tool DAVID (Database for Annotation, Visualization and Integrated Discovery) v6.7 [23] was used to look for functional enrichment of genes with more than 3-fold of change and adjusted p-