

Nrf2 Signaling in Macrophages

Hong Zhu¹, Zhenquan Jia², and Y. Robert Li¹⁻⁴

¹Campbell University Jerry M. Wallace School of Osteopathic Medicine, Buies Creek, NC 27506, USA;

²Department of Biology, University of North Carolina, Greensboro, NC 27412, USA; ³Virginia Tech–Wake Forest University School of Biomedical Engineering and Sciences, Blacksburg, VA 24061, USA; ⁴Department of Biomedical Sciences and Pathobiology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA

Correspondence: zhu@campbell.edu (H.Z.)

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ABSTRACT | The nuclear factor E2-related factor 2 (Nrf2) is known as the chief regulator of cellular antioxidant defenses as well as a suppressor of inflammation. Macrophages act as major players in inflammatory responses. Because oxidative stress and inflammation are two intertwined processes, the anti-inflammatory activity of Nrf2 signaling is believed to result from its upregulation of cellular antioxidant defenses via the antioxidant response element-driven transcription. In a recent article published in *Nature Communications* (May 23, 2016; doi: 10.1038/ncomms11624), Kobayashi et al. reported that Nrf2 suppresses transcriptional upregulation of pro-inflammatory cytokines independent of its role in regulating cellular antioxidants and redox status. This study by Kobayashi et al. provides novel insights into the molecular basis of Nrf2 acting as a suppressor of inflammation.

KEYWORDS | Inflammation; Macrophages; Nrf2; Redox signaling

ABBREVIATIONS | Nrf2, nuclear factor E2-related factor 2; NF-κB, nuclear factor kappa B; ROS, reactive oxygen species

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1. ANTI-INFLAMMATORY FUNCTION OF NRF2: REDOX-DEPENDENT PATHWAY

Inflammation and oxidative stress are closely intertwined. On the one hand, activation of inflammatory

cells, especially phagocytic cells results in the production of large amounts of reactive oxygen and nitrogen species (ROS/RNS) causing oxidative stress and tissue injury, as well as cancer [1, 2]. Such an oxidative tissue injury represents a major pathophys-

iological process of inflammatory disorders. On the other hand, ROS, due to their redox properties, activate transcription factors and intracellular signaling cascades, especially nuclear factor kappa B (NF- κ B) and mitogen-activated protein kinases, leading to the increased transcription of pro-inflammatory cytokines and the consequent perpetuation of inflammatory responses [3, 4].

Because of the above relationship, it is understandable that antioxidants possess anti-inflammatory activities, at least, to some extent. It is also for the same rationale that the nuclear factor E2-related factor 2 (Nrf2), a central regulator of antioxidant genes, is widely viewed as an anti-inflammatory molecule. Indeed, Nrf2 deficiency aggravates the pathophysiology of inflammatory disorders [5], and on the contrary, Nrf2 activation frequently results in the suppression of inflammatory stress and the consequent tissue injury [6].

2. ANTI-INFLAMMATORY FUNCTION OF NRF2: REDOX-INDEPENDENT PATHWAY

Nrf2 plays a central role in regulating cellular antioxidant and other cytoprotective genes, in cells including macrophages [7, 8]. Numerous studies also demonstrate that this transcription factor appears to also modulate the expression of genes whose products have little or nothing to do with oxidative stress [9, 10]. It is not surprising for a particular transcription factor to be involved in the regulation of distinct classes of genes. In this context, a fundamental question about Nrf2 is that can its anti-inflammatory function be independent of its role in directly up-regulating antioxidant genes?

In a recent study appeared in *Nature Communications*, Kobayashi et al. reported that Nrf2 suppresses macrophage inflammatory responses through blocking proinflammatory cytokine transcription [11]. Using bone marrow-derived macrophages, these authors first demonstrated that Nrf2 inhibited the induction by lipopolysaccharide and interferon- γ of a subset of proinflammatory genes [11]. With Nrf2 ChIP-seq and ChIP-qPCR analyses, Kobayashi et al. then showed the binding of Nrf2 to the proximity of proinflammatory genes of interleukin-6 (IL-6) and interleukin-1 α and β . They went on to further demonstrate that such Nrf2 binding directly inhibits the induced expression of the above cytokine

genes [11]. Notably, the Nrf2-mediated inhibition of the proinflammatory cytokine genes is independent of the cellular redox status, further supporting the notion that the transcriptional inhibition of the cytokine genes is a primary action of Nrf2, rather than a secondary effect caused by the elimination of ROS through the production of Nrf2-regulated antioxidant enzymes and proteins [11].

To decipher the underlying molecular mechanism, Kobayashi et al. elegantly demonstrated that Nrf2 inhibits the recruitment of RNA polymerase II onto the proinflammatory cytokine gene loci without affecting the NF- κ B p65 recruitment, and the inhibition occurs at the transcription initiation step [11]. NF- κ B is a critical transcriptional regulator of many proinflammatory cytokine genes [12]. The no effect on NF- κ B p65 recruitment suggests the involvement of other machineries in the Nrf2-mediated transcriptional suppression. Additional experiments by Kobayashi et al. also showed Nrf2-mediated inhibition of proinflammatory cytokine genes is independent of the antioxidant response element in upstream regulatory region [11].

To determine the *in vivo* significance, Kobayashi et al. employed the whole mount *in vivo* monitoring system with human IL-6-luciferase reporter to successfully verify the Nrf2-mediated inhibition of IL-6 gene induction as well as inflammatory symptoms in live mice [11].

3. CONCLUSION AND PERSPECTIVES

Although the detailed molecular machineries remain to be delineated, the study by Kobayashi et al. identifies Nrf2 as a direct suppressor of transcriptional induction of certain proinflammatory cytokine genes. This novel finding revealed a distinct mechanism by which Nrf2 signaling acts to inhibit inflammation and inflammatory tissue injury. It thus appears that Nrf2 may control inflammation through both antioxidant (redox)-dependent and -independent mechanisms (**Figure 1**). The differential contribution of these two distinct mechanisms to the anti-inflammatory function may vary with different cell types or inflammatory stimuli. Nevertheless, identification of the transcriptional suppression of certain inflammatory cytokine genes by Nrf2 in macrophages would certainly prompt more research efforts into deciphering the molecular basis of Nrf2 function in

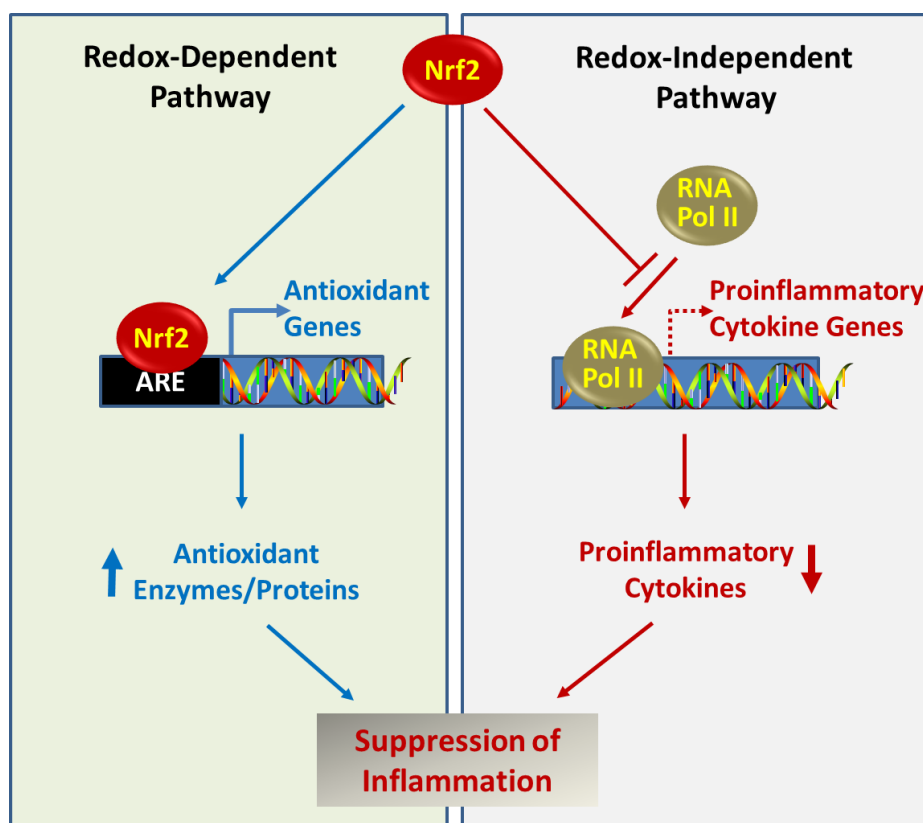


FIGURE 1. The two faces of Nrf2 in suppressing inflammation. As illustrated, Nrf2 signaling may suppress inflammation via two pathways: redox-dependent and -independent pathways. RNA pol II denotes RNA polymerase II; ARE denoted antioxidant response element. This scheme is based on Ref. [9].

cell physiology and pathophysiology. Future work is warranted to determine the transcriptional suppression by Nrf2 signaling of other genes whose products are involved in inflammatory disorders as well as other pathophysiological conditions.

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