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New insights into nuclear factor erythroid 2-related factors in toxicology and pharmacology

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1 **New Insights into Nuclear factor erythroid 2-related factors in**
2 **Toxicology and Pharmacology**

3 The cap'n'collar-basic region leucine zipper (CNC-bZIP) family of transcription factors
4 includes the founding member Nuclear factor-erythroid 2 (NF-E2) p45, NF-E2 p45-related factor
5 1 (Nrf1; also known as NFE2L1, LCRF1, TCF11, HBZ17 or FLJ00380), Nrf2 and Nrf3 (also
6 abbreviated as NFE2L2 and NFE2L3, respectively), and the more distantly related members BTB
7 (i.e., Broad complex, Tramtrack, and Bric-à-Brac) domain and CNC homolog 1 (BACH1) and
8 BACH2 (Tebay *et al.*, 2015; Katsuoka and Yamamoto, 2016; Zhang and Xiang, 2016; Zhu *et al.*,
9 2016; Yamamoto *et al.*, 2018). In the last two decades, our understanding of CNC-bZIP proteins
10 has advanced enormously, and our appreciation of their physiological significance has similarly
11 increased. The primary objective of this special issue (SI) is to stimulate continuing effort to
12 understand the toxicological and pharmacological roles of CNC-bZIP proteins, and that of Nrf2
13 and Nrf1 in particular.

14 Nrf2 is normally found in the cytoplasm of mammalian cells, where it associates with the
15 redox-sensitive Kelch-like ECH-associated protein 1 (Keap1) E3 ubiquitin ligase substrate
16 adaptor that polyubiquitinylates Nrf2 and targets it for proteolytic degradation by the 26S
17 proteasome. This mechanism keeps cellular Nrf2 levels low and prevents Nrf2 accumulation in
18 the nucleus where it would mediate signaling effects (Suzuki and Yamamoto, 2017; Yamamoto *et*
19 *al.*, 2018). In response to a wide variety of oxidative and electrophilic insults, Nrf2 avoids Keap1-
20 mediated proteolytic digestion and accumulates in the nucleus where it heterodimerizes with
21 small musculoaponeurotic fibrosarcoma (MAF) proteins and binds to antioxidant response
22 element (ARE; 5'-TGACNNNGC-3') sequences within target genes, resulting in expression of
23 that gene for a limited period (Suzuki and Yamamoto, 2017; Yamamoto *et al.*, 2018). The target

24 genes of Nrf2 include those that encode a variety of antioxidant and detoxification enzymes. Thus,
25 the Keap1-Nrf2 system is recognized as a key player in controlling biochemical defense against
26 exogenous and endogenous electrophilic and oxidative stressors. Importantly, accumulating
27 evidence indicates that Nrf2 also plays critical roles in regulating expression of numerous genes
28 involved in cell metabolism, proliferation and differentiation ((Pi *et al.*, 2010; Xue *et al.*, 2013;
29 Murakami and Motohashi, 2015).

30 In the Review section of this SI, Ryoo and Kwak revisited recent experimental observations
31 on the relationship between Nrf2 and mitochondria and discussed mechanisms by which Nrf2
32 controls mitochondria and metabolism in cancer cells. These authors report that Nrf2 is positively
33 associated with mitochondrial biogenesis through direct upregulation of mitochondrial
34 transcription factors and is involved in the mitochondrial quality control system via activation of
35 mitophagy. Additionally, Nrf2 modulation in cancer cells leads to changes in the mitochondrial
36 respiratory system and cancer bioenergetics that overall affect cancer metabolism. Ikehata and
37 Yamamoto reviewed recent progress in the study of contributions by Nrf2 and related factors to
38 protection against ultraviolet radiation (UVR). The Keap1-Nrf2 system is not always efficient in
39 responding to UVR, especially to short wavelengths such as UVC and UVB, indicating that UVR
40 is a poor activator of the Keap1-Nrf2 system. However, sustained activation of Nrf2 appears to
41 suppress the harmful effects of chronic UVR exposure, such as photoaging and carcinogenesis in
42 the skin, indicating that Nrf2 activation is beneficial for the protection of the skin from the
43 harmful effects of UVR. However, sustained activation of Nrf2 may also adversely affect the skin,
44 especially in the case of UVR-induced carcinogenesis. Sun *et al.* assessed the roles of Nrf2 in the
45 development of alcoholic liver disease (ALD) and emphasized that Nrf2 in different cell types in
46 the liver may play paradoxical roles in the progression of ALD. In the early stages of ALD, Nrf2
47 in hepatocytes plays a crucial role in regulating redox balance and lipid metabolism. With the

48 progression to steatohepatitis, the role of Nrf2 in Kupffer cells become evident, which alleviates
49 the inflammatory response in the liver. During end-stage ALD, Nrf2 in hepatic stellate cells may
50 be critical in modulating fibrogenesis. In light of the important protective roles of Nrf2 against
51 oxidative damage, the study and validation of possible pharmacological targets that would restore
52 the coordination of the networks in related pathologies has recently received particular attention.
53 In the review by Yamawaki *et al.*, they summarized the current issues in the treatment of kidney
54 diseases, Nrf2 activators as treatment options, and perspectives on pharmaceutical applications of
55 Nrf2 activators.

56 In the Research Articles section of this SI, Raghunath *et al.* identified ARE sequences in all
57 protein-coding genes in the zebrafish genome. They found multiple unique AREs that have not
58 been reported previously in cytoprotective genes of this organism. In a detailed mechanistic study,
59 McMahon *et al.* uncovered that Keap1 directly senses Zn^{2+} through a cluster of amino-acids that
60 include His-225, Cys-226 and Cys-613. They presented evidence that binding of Zn^{2+} triggers a
61 conformational switch in Keap1, which is envisaged to perturb the architecture of the cullin-3
62 RING ubiquitin ligase (CRL) complex CRL^{Keap1} , such that bound Nrf2 becomes mis-aligned with
63 respect to the ubiquitin-charged E2 enzyme. The data are consistent with the notion that Keap1
64 possesses a Zn^{2+} sensor whose triggering distorts its structure in a fashion that inhibits
65 ubiquitylation of Nrf2 upon the CRL^{Keap1} complex. Chen's group explored the role of Nrf2 in
66 mediating aberrant hematopoiesis in response to low-dose benzene exposure in *Nrf2*-KO mice.
67 They found that the hematotoxicity of low-dose benzene seems to decrease in *Nrf2*-KO mice
68 based on peripheral blood cell counts, despite the fact that oxidative and DNA damage was
69 significantly enhanced in the mutant mice. In addition, deficiency of Nrf2 triggered proliferation
70 and differentiation of hematopoietic cells by accelerating cell cycle progression and induced a
71 morphological abnormality in peripheral erythrocytes and bone marrow cells, implicating

72 compensatory changes that allow induction of dysfunctional defective blood cells. In an *in vitro*
73 study, Meng's group reported that rat primary microglia and astrocytes display different
74 responses to lead toxicity. In another *in vitro* study, Zhao's group examined the role of the Nrf2
75 signaling pathway in the cytotoxicity induced by the hypoxia mimetic cobalt chloride (CoCl₂) in
76 human keratinocyte HaCaT cells. These workers found that stable knockdown (KD) of Nrf2
77 dramatically reduced expression of antioxidant enzymes and sensitized the cells to acute CoCl₂-
78 induced oxidative stress and cytotoxicity, whereas *Keap1*-KD cells showed enhanced expression
79 of ARE-driven genes and resistance to CoCl₂-induced cell damage. In addition, pretreatment of
80 HaCaT cells with *tert*-butylhydroquinone protected these cells from CoCl₂-induced cell injury in
81 an Nrf2-dependent fashion. In a systematic *in vivo* study, Cho *et al.* demonstrated that
82 sulforaphane (SFN) significantly reduced acute lung injury-like phenotypes caused by subsequent
83 hyperoxia exposure in an Nrf2-dependent manner. Differential lung transcriptome changes
84 induced by SFN in wildtype and *Nrf2*-KO mice suggested that it acts through Nrf2 enhancing
85 pulmonary mitochondrial dynamics and metabolism to maintain the bioenergetic demands of lung
86 cells against oxidative stress. As a part of a series of studies on ARE inhibitors from Pi's
87 laboratory, Zhu *et al.* identified a traditional Chinese medicine, triptolide, as an **effective and**
88 **potent** Nrf2-ARE inhibitor. Importantly, triptolide, at non-toxic levels, markedly sensitized non-
89 small-cell lung cancer cells to chemotherapeutic treatments *in vitro* and in a xenograft mouse
90 tumor model.

91 Nrf1 serves as a unique vital player in maintaining cellular homeostasis and organ integrity
92 during normal development and cell growth throughout life. Global loss of Nrf1 results in severe
93 oxidative stress, genomic instability, embryonic lethality and developmental disorders.
94 Conditional knockout of Nrf1 results in adult diseases such as non-alcoholic steatohepatitis,
95 hepatocellular carcinoma, pancreatic β -cell and adipocyte dysfunction and neurogenerative

96 diseases (Pi *et al.*, 2010; Xue *et al.*, 2013; Murakami and Motohashi, 2015; Zheng *et al.*, 2015;
97 Kim *et al.*, 2016; Fu *et al.*, 2018; Hou *et al.*, 2018; Wang *et al.*, 2018). Thus, Nrf1 is critically
98 implicated in a variety of important physio-pathological processes by governing expression of
99 crucial genes in order to reinforce antioxidant, detoxification and cytoprotective responses to
100 cellular stress. Of clinical interest, Nrf1 mediates the proteasomal 'bounce-back' response,
101 leading to drug resistance to proteasomal inhibitors for clinical treatment of neuroblastoma,
102 multiple myeloma and triple-negative breast cancers (Steffen *et al.*, 2010; Bugno *et al.*, 2015;
103 Sekine *et al.*, 2018). During its translation, Nrf1 is targeted to the endoplasmic reticulum (ER)
104 and subject to extensive post-translational modification before it regulates its target genes.
105 However, the mechanisms whereby Nrf1 is processed and topologically released from the ER
106 before entering the nucleus is hotly debated. In this SI, a series of experiments from Zhang's
107 laboratory demonstrate the maturation processing of Nrf1 to remove its N-terminal ~12.5-kDa
108 and longer polypeptides. The authors have further elucidated topo-vectorial mechanisms that
109 monitor dynamic movement of Nrf1 in and out of the ER lumen, as well as the selective
110 proteolytic processing of the CNC-bZIP protein to remove distinct lengths of its NTD (most of
111 which was refolded as a UBL module) and PEST-adjointing AD1 domains. More importantly,
112 they have also established a general criterion acceptable for identification of the endogenous
113 Nrf1 α /TCF11 and derivative isoforms, with distinct molecular weights and half-lives determined
114 in various experimental cellular settings. Furthermore, they propose that coupled positive and
115 negative feedback circuits exist between Nrf1 and its target genes. These results suggest Nrf1 is
116 subject to dual opposing control in which low doses of proteasomal inhibitors elicit a 'bounce-
117 back' response but higher doses inhibit the transcription factor. Collectively, the findings from
118 Zhang's group suggest the potential of Nrf1 to be developed as a new target for chemoprevention
119 and therapy of cancers and other diseases.

120 Coordinately dealing with fluctuating levels of numerous metabolic and environmental
121 stresses is critical for the survival of cells and whole organisms. Perturbations in redox balance
122 may impair cellular homeostasis and trigger the onset of disease. Accordingly, cells have
123 developed multiple and well-conserved mechanisms that regulate adaptive antioxidant responses.
124 Oxidative stress, which is defined as a general response to internal and environmental oxidative
125 challenges, is involved in triggering adaptation to oxidative damage. On the other hand, persistent
126 oxidative stress may lead to disruption of redox signaling and loss of homeostatic mechanisms
127 (Zhang *et al.*, 2010; Fu *et al.*, 2016). Thus, precise coordination of cellular adaptive responses to
128 oxidative insults promotes stress resistance and recovery of homeostasis, whereas persistent
129 adaptation would have a cost that may be involved in the pathogenesis of many chronic disorders
130 (see Figure 1).

131 For many decades, ROS were considered cytotoxic waste products arising from cellular
132 processes. Thus, antioxidant interventions were established in the settings of aging and chronic
133 diseases. However, animal studies and epidemiological investigations on the therapeutic
134 outcomes of antioxidant interventions have provided data that contradict the view that ROS are
135 merely of toxicological significance, questioning long-standing beliefs of an ultimate beneficial
136 role for antioxidant therapies in health and disease (Zhang *et al.*, 2010; Fu *et al.*, 2016). In
137 agreement with the aforementioned accumulating evidence and in the context of major chronic
138 diseases, we reasonably hypothesized that persistent activation of Nrf2-ARE caused by
139 environmental stressors may be involved in the pathogenesis of various chronic diseases, such as
140 Type 2 diabetes and malignant tumors (see Figure 1). Of course, coordinated efforts are needed to
141 clarify the exact roles of various isoforms of Nrf2 in the development and intervention of various
142 chronic diseases.

143 In summary, while significant progress has been made in terms of elucidating how the
144 different Nrf transcription factors (in particular Nrf2, and to a much lesser extent Nrf1) regulate
145 the antioxidant response, critical questions still remain. Most obviously, we know relatively little
146 about NF-E2 p45, Nrf3, BACH1 and BACH2. Other important open issues relating to Nrfs in
147 toxicology and pharmacology may include, but are not limited to: (1) determining the spectrum of
148 target genes of Nrfs in different cells under diverse stress challenges, and the potential
149 involvement of Nrfs in regulating genes independently of ARE sequences; (2) the mechanistic
150 aspects of the complex regulatory network of Nrfs-mediated transcription under basal
151 physiological conditions and under adaptive response conditions; (3) the transcriptional
152 regulation of Nrfs under a sustained stress challenge, in particular the involvement of non-coding
153 RNAs, such as miRNAs; (4) the crosstalk between Nrfs and other stress response machinery in
154 response to various internal and environmental challenges; (5) the toxicological significance and
155 application of Nrfs network perturbation in toxicity testing; (6) the characterization of the cell-
156 specific physiological functions of different isoforms of Nrf1; (7) identification and application
157 of novel modulators targeting specifically the transcriptional activity of various isoforms of
158 Nrfs; (8) precise phenotyping of cell-specific knockout or overexpression of Nrfs in a variety of
159 disease models; and (9) identification and characterization of functional SNPs and epigenetic
160 sites in the human genes of Nrfs.

161

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187 **References :**

- 188 Bugno, M., Daniel, M., Chepelev, N. L., and Willmore, W. G. (2015). Changing gears in
189 Nrf1 research, from mechanisms of regulation to its role in disease and
190 prevention. *Biochimica et biophysica acta* **1849**, 1260-1276.
- 191 Fu, J., Hou, Y., Xue, P., Wang, H., Xu, Y., Qu, W., Zhang, Q., and Pi, J. (2016). Nrf2 in
192 Type 2 diabetes and diabetic complications: Yin and Yang *Current Opinion in*
193 *Toxicology* **1**, 9-19.
- 194 Fu, J., Zheng, H., Cui, Q., Chen, C., Bao, S., Sun, J., Li, L., Yang, B., Wang, H., Hou, Y.,
195 Xu, Y., Xu, Y., Zhang, Q., and Pi, J. (2018). Nfe2l1-silenced insulinoma cells
196 acquire aggressiveness and chemoresistance. *Endocrine-related cancer* **25**, 185-
197 200.
- 198 Hou, Y., Liu, Z., Zuo, Z., Gao, T., Fu, J., Wang, H., Xu, Y., Liu, D., Yamamoto, M., Zhu,
199 B., Zhang, Y., Andersen, M. E., Zhang, Q., and Pi, J. (2018). Adipocyte-specific
200 deficiency of Nfe2l1 disrupts plasticity of white adipose tissues and metabolic
201 homeostasis in mice. *Biochemical and biophysical research communications* **503**,
202 264-270.
- 203 Katsuoka, F., and Yamamoto, M. (2016). Small Maf proteins (MafF, MafG, MafK):
204 History, structure and function. *Gene* **586**, 197-205.
- 205 Kim, H. M., Han, J. W., and Chan, J. Y. (2016). Nuclear Factor Erythroid-2 Like 1
206 (NFE2L1): Structure, function and regulation. *Gene* **584**, 17-25.
- 207 Murakami, S., and Motohashi, H. (2015). Roles of Nrf2 in cell proliferation and
208 differentiation. *Free radical biology & medicine* **88**, 168-178.
- 209 Pi, J., Leung, L., Xue, P., Wang, W., Hou, Y., Liu, D., Yehuda-Shnaidman, E., Lee, C.,
210 Lau, J., Kurtz, T. W., and Chan, J. Y. (2010). Deficiency in the nuclear factor E2-
211 related factor-2 transcription factor results in impaired adipogenesis and protects
212 against diet-induced obesity. *The Journal of biological chemistry* **285**, 9292-9300.
- 213 Sekine, H., Okazaki, K., Kato, K., Alam, M. M., Shima, H., Katsuoka, F., Tsujita, T.,
214 Suzuki, N., Kobayashi, A., Igarashi, K., Yamamoto, M., and Motohashi, H.
215 (2018). O-GlcNAcylation Signal Mediates Proteasome Inhibitor Resistance in
216 Cancer Cells by Stabilizing NRF1. *Molecular and cellular biology*.
- 217 Steffen, J., Seeger, M., Koch, A., and Kruger, E. (2010). Proteasomal degradation is
218 transcriptionally controlled by TCF11 via an ERAD-dependent feedback loop.
219 *Molecular cell* **40**, 147-158.
- 220 Suzuki, T., and Yamamoto, M. (2017). Stress-sensing mechanisms and the physiological
221 roles of the Keap1-Nrf2 system during cellular stress. *The Journal of biological*
222 *chemistry* **292**, 16817-16824.
- 223 Tebay, L. E., Robertson, H., Durant, S. T., Vitale, S. R., Penning, T. M., Dinkova-
224 Kostova, A. T., and Hayes, J. D. (2015). Mechanisms of activation of the
225 transcription factor Nrf2 by redox stressors, nutrient cues, and energy status and
226 the pathways through which it attenuates degenerative disease. *Free radical*
227 *biology & medicine* **88**, 108-146.

228 Wang, H., Zhu, J., Liu, Z., Lv, H., Lv, P., Chen, F., Fu, J., Hou, Y., Zhao, R., Xu, Y.,
229 Zhang, Q., and Pi, J. (2018). Silencing of long isoforms of nuclear factor
230 erythroid 2 like 1 primes macrophages towards M1 polarization. *Free radical*
231 *biology & medicine* **117**, 37-44.

232 Xue, P., Hou, Y., Chen, Y., Yang, B., Fu, J., Zheng, H., Yarborough, K., Woods, C. G.,
233 Liu, D., Yamamoto, M., Zhang, Q., Andersen, M. E., and Pi, J. (2013). Adipose
234 deficiency of Nrf2 in ob/ob mice results in severe metabolic syndrome. *Diabetes*
235 **62**, 845-854.

236 Yamamoto, M., Kensler, T. W., and Motohashi, H. (2018). The KEAP1-NRF2 System: a
237 Thiol-Based Sensor-Effector Apparatus for Maintaining Redox Homeostasis.
238 *Physiological reviews* **98**, 1169-1203.

239 Zhang, Q., Pi, J., Woods, C. G., and Andersen, M. E. (2010). A systems biology
240 perspective on Nrf2-mediated antioxidant response. *Toxicol Appl Pharmacol* **244**,
241 84-97.

242 Zhang, Y., and Xiang, Y. (2016). Molecular and cellular basis for the unique functioning
243 of Nrf1, an indispensable transcription factor for maintaining cell homeostasis
244 and organ integrity. *The Biochemical journal* **473**, 961-1000.

245 Zheng, H., Fu, J., Xue, P., Zhao, R., Dong, J., Liu, D., Yamamoto, M., Tong, Q., Teng,
246 W., Qu, W., Zhang, Q., Andersen, M. E., and Pi, J. (2015). CNC-bZIP protein
247 Nrf1-dependent regulation of glucose-stimulated insulin secretion. *Antioxid Redox*
248 *Signal* **22**, 819-831.

249 Zhu, J., Wang, H., Chen, F., Fu, J., Xu, Y., Hou, Y., Kou, H. H., Zhai, C., Nelson, M. B.,
250 Zhang, Q., Andersen, M. E., and Pi, J. (2016). An overview of chemical inhibitors
251 of the Nrf2-ARE signaling pathway and their potential applications in cancer
252 therapy. *Free radical biology & medicine* **99**, 544-556.
253

254 **Figure legends :**

255 **Figure 1. Nrfs may play paradoxical *Yin-and-Yang* roles in the development of oxidative**
256 **stress-related disorders.** *Yin*-side of ROS: Prolonged overproduction of ROS may result in
257 oxidative damage and even cell death, leading to impaired cell function; *Yang*-side of ROS:
258 Transient ROS production in response to various stimuli may function as signals mediating
259 cellular responses. *Yin*-side of antioxidants: Antioxidants may blunt ROS signaling in the cell;
260 *Yang*-side of antioxidants: Antioxidants may generally protect cells against oxidative damage.
261 *Yin*-side of Nrfs: Chronic activation of Nrfs-mediated antioxidant response under persistent
262 oxidative stress may blunt normal ROS signaling in the cell; *Yang*-side of Nrfs: Nrfs activation
263 and subsequent induction of antioxidants may protect cells from oxidative damage.