ROS

RESEARCH HIGHLIGHTS

Nrf2: Its Newly Discovered Machineries

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ABSTRACT | Since the discovery of its role in regulating phase 2 genes in 1997, Nrf2 has become a superstar among redox-regulated transcription factors. Research has continued to shed new light on the nature of this signaling molecule and its associated machineries in cell physiology and pathophysiology. This ROS Research Highlights article summarizes several major research discoveries on Nrf2 that were reported in Cell and Nature over the past few months. These include the novel findings on: (1) Nrf2–Pitx2 in cardiac repair; (2) progerin–Nrf2 in aging; (3) cilium–autophagy–Nrf2 in stem cell biology; and (4) EGFR–Nrf2 in pancreatic cancer maintenance. These novel observations greatly advance our current understanding of the cell biology of Nrf2 signaling and provide insights into the ongoing efforts in developing mechanistically based strategies for disease intervention via selectively targeting Nrf2 along with its associated machineries.

KEYWORDS | Aging; Autophagy; Cardiac repairing; Cilium; Epithelial growth factor; Nrf2 signaling; Pancreatic cancer; Pitx2; Progerin; Reactive oxygen species; Stem cell

ABBREVIATIONS | ARE, antioxidant response element; EGFR, epithelial growth factor receptor; hESC, human embryonic stem cell; HGPS, Hutchinson–Gilford progeria syndrome; iPSC, induced pluripotent stem cell; ROS, Reactive oxygen species

CONTENTS

1. NRF2–PITX2 IN CARDIAC REPAIR

Understanding the molecular process of cardiac repair is instrumental in devising strategies for treating cardiac diseases, such as myocardial infarction and heart failure. The homobox transcription factor Pitx2 has essential roles in the development of different organs including the heart [1]. In a recent article published in the June 2 (2016) issue of Nature, Tao et al. elegantly demonstrated that Pitx2-deficient neonatal

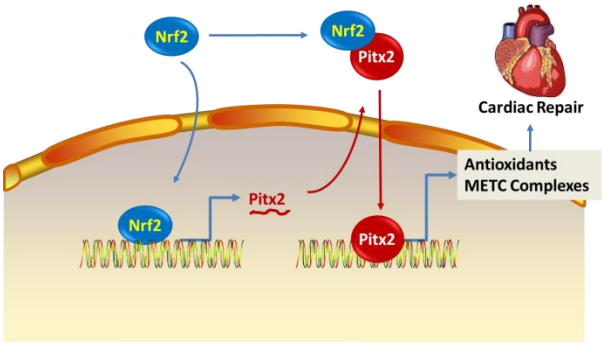

FIGURE 1. Nrf2 induces Pitx2 expression and nuclear translocation, leading to activation of cardiac repair mechanism. See Section 1 for more description. Based on Ref. [2].

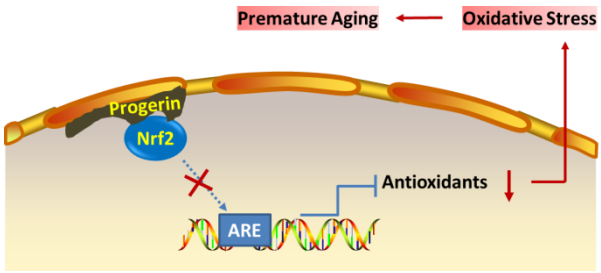

FIGURE 2. Progerin sequesters Nrf2 and causes its subnuclear mislocalization, resulting in decreased antioxidants and subsequent oxidative stress and premature aging. See Section 2 for more description. Based on Ref. [5].

mouse hearts failed to repair following apex resection, and on the other hand, adult mouse cardiomyocytes with Pitx2 gain-of-function efficiently regenerated following myocardial infarction [2]. They further showed that Pitx2 activated genes encoding mitochondrial electron transport chain components and antioxidant enzymes [2]. Importantly, Tao et al. found that Nrf2, the central regulator of cellular antioxidant genes, also directly regulated the expression of Pitx2. Moreover, Nrf2 was found to bind to Pitx2, causing its nuclear translocation [2]. In line with the ability of Pitx2 to upregulate myocardial antioxidant genes (e.g., superoxide dismutase, glutathione peroxidase), Pitx2 mutant myocardium had increased levels of reactive oxygen species (ROS), while supplementation of *N*-acetylcysteine suppressed the Pitx2 loss-of-function phenotype as evidenced by the decreased size of the myocardial scar [2]. Collectively, the above novel findings by Tao et al. advance our knowledge on Nrf2 by demonstrating that this redox regulator also controls the expression as well as the nuclear translocation of a transcription factor (i.e., Pitx2) that is essential for cardiac repair following myocardial infarction in an animal model.

This discovery may have important implications. Pharmacological activation of Nrf2 has been demonstrated to be protective in a variety of disease models, including myocardial ischemia-reperfusion injury [3, 4]. It is imperative to investigate if pharmacological stimulators of Nrf2 signaling also promote Pitx2-regulated cardiac repair following myocardial infarction (**Figure 1**).

2. PROGERIN–NRF2 IN AGING

Progerin, a mutant form of the nuclear architectural protein—lamin A, causes a rare, but severe premature aging syndrome, known as Hutchinson–Gilford progeria syndrome (HGPS). In an article appeared in the June 2 (2016) issue of Cell, Kubben et al. reported repression of Nrf2 signaling as a driver mechanism in HGPS [5]. Kubben et al. elegantly demonstrated that progerin could bind to and sequester Nrf2 and cause its subnuclear mislocalization in a cellular model [5]. This mislocation led to impaired Nrf2 transcriptional activity, thereby causing decreased expression of cellular antioxidant genes and the consequent accumulation of ROS and oxidative stress. By generating induced pluripotent stem cells (iPSCs) from HGPS patient fibroblasts, Kubben et al. further showed that Nrf2 pathway activation restored in vivo viability of HGPS mesenchymal stem cells [5]. In this regard, Kubben et al. used an established animal model assay system, in which mesenchymal stem cells were implanted into the tibialis anterior muscle and engraftment and survival were measured. Notably, pharmacological activation of Nrf2 by oltipraz, a dithiolethione compound, was also found to be effective in restoring in vivo viability of HGPS mesenchymal stem cells [5]. This new discovery highlights the feasibility of using pharmacological agents to activate Nrf2 signaling to treat premature aging in HGPS as well as for the intervention of other pathophysiological conditions that affect the normal aging process (**Figure 2**).

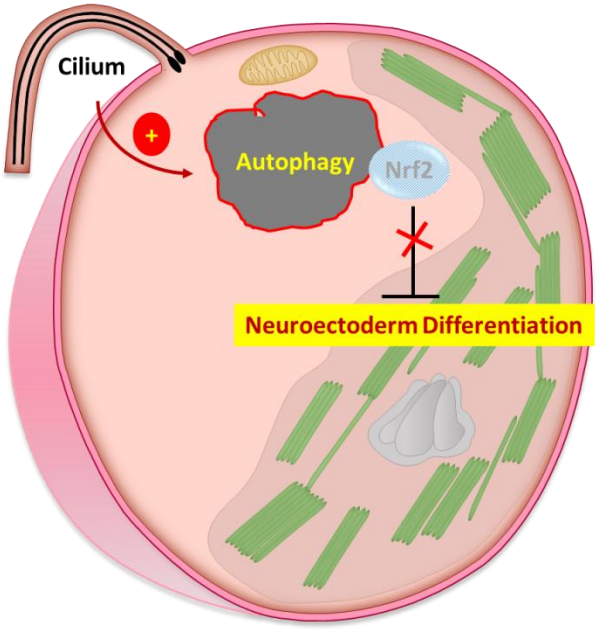

FIGURE 3. Cilium inactivates Nrf2 via autophagy, permitting neuroectoderm differentiation. See Section 3 for more description. Based on Ref. [9].

3. CILIUM–AUTOPHAGY–NRF2 IN STEM CELL BIOLOGY

A previous ROS Research Highlights article discussed an essential role for Nrf2 silencing in neuron maturation [6]. Multiple studies also demonstrated a role for Nrf2 signaling in regulating stem cell self-renewal [7, 8]. A recent study reported in the April 7 (2016) issue of Cell discovered a new function for Nrf2 signaling in regulating human embryonic stem cell (hESC) differentiation to neuroectoderm [9]. In their study, Jang et al. first showed that Nrf2 regulated early lineage specification by directly controlling OCT4 and NANOG expression in hESCs and then went on to show that Nrf2 repressed neuroectoderm fate by directly controlling OCT4 and NANOG expression and that Nrf2 suppression was a prerequisite for successful neuroectoderm induction. Using iPSCs, Jang et al. demonstrated that Nrf2 activity levels were highly predictive of neuroectoderm differentiation potential in contrast to core pluripotency gene products. Nrf2 inhibition was also sufficient to rescue poorly neurogenic iPSC lines. Considering that autophagy plays an important role in controlling Nrf2 activity in other cellular systems [10, 11], Jang et al. then determined if lineage-dependent autophagy also played a role in regulating Nrf2 activity. They found that autophagy was activated in a lineage-specific manner and controlled early hESC differentiation to neuroectoderm by downregulating Nrf2 activity. They went on to show that the primary cilium was essential for the activation of autophagy during neuroectoderm differentiation. This led Jang et al. to propose that the primary cilium–autophagy–Nrf2 axis coupled to cell-cycle progression dictated the differentiation of hESCs to neuroectoderm. It is noteworthy that the primary cilium is a microtubule-based antenna-like organelle that emanates from the surface of virtually all cells in the mammalian body. This organelle plays an important role in cell sensing and signaling, thereby the regulation of cell growth and differentiation [12]. The study by Jang et al. thus provided another example of the increasingly complex machineries involving Nrf2 signaling in stem cell biology (**Figure 3**).

4. EGFR–NRF2 IN PANCREATIC CANCER MAINTENANCE

Nrf2 plays complex roles in cancer [13, 14]. On the one hand, Nrf2 signaling protects against chemical carcinogenesis by promoting the detoxification of chemical carcinogens via primarily upregulating phase 2 and other cytoprotective genes [15]. On the other hand, multiple studies suggest that constitutive overexpression/activation of Nrf2 may promote cancer cell growth and metastasis by providing a more favorable redox environment for cancer cells as well as by causing cancer drug resistance [16, 17]. Indeed, pharmacologically elevating ROS in cancer cells has been employed as a promising strategy for cancer treatment. In this context, cancer cells typically have higher levels of ROS compared to normal cells and are more susceptible to additional ROS-mediated cell killing [18]. How exactly Nrf2 promotes cancer development remains unclear. In the August 11 (2016) issue of Cell, Chio et al. reported their findings that Nrf2 promoted tumor maintenance by modulating mRNA translation in pancreatic cancer [19]. Using Kras mutant pancreatic epithelial cells, Chio et al. first demonstrated that Nrf2 regulated redox homeostasis and cell proliferation, and ablation of Nrf2 impeded cell proliferation, which could be reversed by

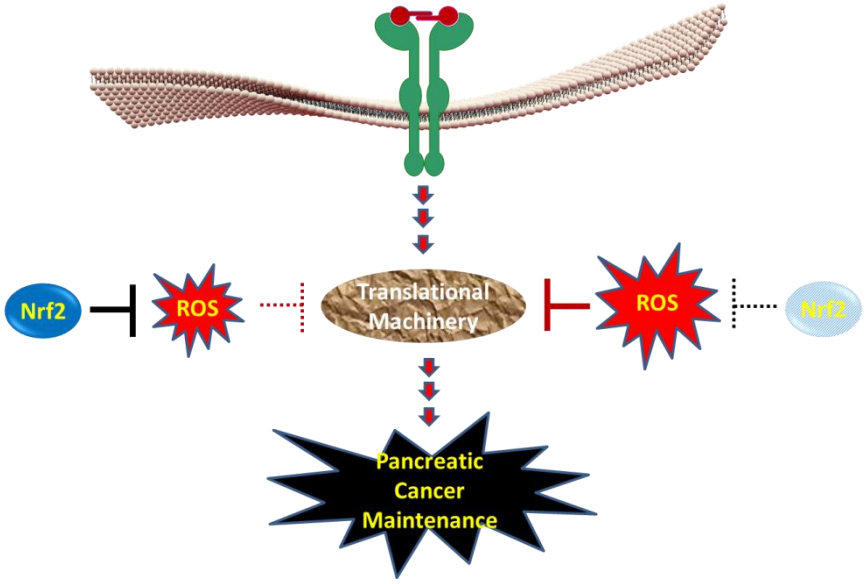

FIGURE 4. Nrf2 controls ROS-mediated inactivation of translational machinery in pancreatic cancer. See Section 4 for more description. Based on Ref. [19].

treatment with *N*-acetylcysteine [19], an antioxidant compound that augments intracellular levels of reduced form of glutathione (GSH). This suggested the critical involvement of an oxidative mechanism in the inhibition of the Kras mutant cell proliferation by Nrf2 deficiency. Chio et al. then performed global cysteine proteomic studies, which revealed the redox dependent regulation of the transcriptional machinery and that Nrf2 exercised redox-dependent control over multiple aspects of the translational machinery [19]. They went on to further demonstrate that epithelial growth factor receptor (EGFR) signaling pathways upstream of Cap-dependent translation were impaired in Nrf2-deficient cells and that Nrf2 supported Cap-dependent translation of pro-survival transcripts [19]. Notably, combined inhibition of AKT by MK2260 and depletion of cellular GSH by buthionine sulfoximine blunted pancreatic cancer growth and survival in an animal model [19]. Collectively, these novel findings by Chio et al. demonstrated translational machinery as an important target of redox modulation by Nrf2 signaling in the control of pancreatic cancer maintenance and suggested that Nrf2 could serve as an important molecular target for developing therapeutic modalities for the management of pancreatic cancer (**Figure 4**).

5. CONCLUSION AND PERSPECTIVES

Nrf2 as a chief regulator of cellular redox homeostasis has received extensive attention over the past few years regarding its involvement in both physiology and pathophysiology. On the one hand, Nrf2 signaling appears to protect normal cells against oxidative injury, thereby providing a physiologically survival force with desired sequela. On the other hand, this survival force provided by Nrf2 signaling may also operate to promote cancer cell growth and metastasis as well as resistance of cancer cells to drug therapy. Although the biology of Nrf2 may lie primarily in its role in redox regulation, it should be borne in mind that Nrf2 signaling may also function in a redox-independent manner. In addition to binding to the antioxidant response element (ARE) to cause transactivation of antioxidant genes, Nrf2 may also bind to the regulatory regions of other genes via an ARE-independent mechanism. Indeed, a recent study suggested that Nrf2 bound to the proximity of pro-inflammatory cytokine genes (e.g., interleukin-1beta and interleukin-6) in macrophages to block RNA Pol II recruitment and the subsequent transcription of these genes. This translational inhibition was independent of Nrf2 binding to its conventional cis-

element ARE and independent of cellular ROS levels [20]. The continued discoveries of new machineries associated with Nrf2 signaling would certainly not only deepen our understanding of this protein molecule, but also enhance our ability to devise novel therapies to treat human disorders that involve an Nrf2-dependent mechanism.

REFERENCES

1. Franco D, Campione M. The role of Pitx2 during cardiac development: linking left-right signaling and congenital heart diseases. *Trends Cardiovasc Med* 2003; 13(4):157–63.
2. Tao G, Kahr PC, Morikawa Y, Zhang M, Rahmani M, Heallen TR, et al. Pitx2 promotes heart repair by activating the antioxidant response after cardiac injury. *Nature* 2016; 534(7605):119–23. doi: 10.1038/nature17959.
3. Calvert JW, Jha S, Gundewar S, Elrod JW, Ramachandran A, Pattillo CB, et al. Hydrogen sulfide mediates cardioprotection through Nrf2 signaling. *Circ Res* 2009; 105(4):365–74. doi: 10.1161/CIRCRESAHA.109.199919.
4. Ashrafian H, Czibik G, Bellahcene M, Aksentijevic D, Smith AC, Mitchell SJ, et al. Fumarate is cardioprotective via activation of the Nrf2 antioxidant pathway. *Cell Metab* 2012; 15(3):361–71. doi: 10.1016/j.cmet.2012.01.017.
5. Kubben N, Zhang W, Wang L, Voss TC, Yang J, Qu J, et al. Repression of the antioxidant NRF2 pathway in premature aging. *Cell* 2016; 165(6):1361–74. doi: 10.1016/j.cell.2016.05.017.
6. Hopkins RZ. Nrf2 silencing for neuron maturation. *Reactive Oxygen Species* 2016; 1(1):53–8. doi: 10.20455/ros.2016.809
7. Paul MK, Bisht B, Darmawan DO, Chiou R, Ha VL, Wallace WD, et al. Dynamic changes in intracellular ROS levels regulate airway basal stem cell homeostasis through Nrf2-dependent Notch signaling. *Cell Stem Cell* 2014; 15(2):199–214. doi: 10.1016/j.stem.2014.05.009.
8. Tsai JJ, Dudakov JA, Takahashi K, Shieh JH, Velardi E, Holland AM, et al. Nrf2 regulates haematopoietic stem cell function. *Nat Cell Biol* 2013; 15(3):309–16. doi: 10.1038/ncb2699.
9. Jang J, Wang Y, Lalli MA, Guzman E, Godshalk SE, Zhou H, et al. Primary cilium-autophagy-Nrf2 (PAN) axis activation commits human embryonic stem cells to a neuroectoderm fate. *Cell* 2016; 165(2):410–20. doi: 10.1016/j.cell.2016.02.014.
10. Komatsu M, Kurokawa H, Waguri S, Taguchi K, Kobayashi A, Ichimura Y, et al. The selective autophagy substrate p62 activates the stress responsive transcription factor Nrf2 through inactivation of Keap1. *Nat Cell Biol* 2010; 12(3):213–23. doi: 10.1038/ncb2021.
11. Ichimura Y, Waguri S, Sou YS, Kageyama S, Hasegawa J, Ishimura R, et al. Phosphorylation of p62 activates the Keap1-Nrf2 pathway during selective autophagy. *Mol Cell* 2013; 51(5):618–31. doi: 10.1016/j.molcel.2013.08.003.
12. Gerdes JM, Davis EE, Katsanis N. The vertebrate primary cilium in development, homeostasis, and disease. *Cell* 2009; 137(1):32–45. doi: 10.1016/j.cell.2009.03.023.
13. Satoh H, Moriguchi T, Saigusa D, Baird L, Yu L, Rokutan H, et al. NRF2 intensifies host defense systems to prevent lung carcinogenesis, but after tumor initiation accelerates malignant cell growth. *Cancer Res* 2016; 76(10):3088–96. doi: 10.1158/0008-5472.CAN-15-1584.
14. Zhu H, Jia Z, Trush MA, Li YR. Nrf2 deficiency promotes melanoma growth and lung metastasis. *Reactive Oxygen Species* 2016; 2(4):308–14. doi: 10.20455/ros.2016.853
15. Ramos-Gomez M, Kwak MK, Dolan PM, Itoh K, Yamamoto M, Talalay P, et al. Sensitivity to carcinogenesis is increased and chemoprotective efficacy of enzyme inducers is lost in nrf2 transcription factor-deficient mice. *Proc Natl Acad Sci USA* 2001; 98(6):3410–5. doi: 10.1073/pnas.051618798.
16. DeNicola GM, Karreth FA, Humpton TJ, Gopinathan A, Wei C, Frese K, et al. Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. *Nature* 2011; 475(7354):106–9. doi: 10.1038/nature10189.
17. Oshimori N, Oristian D, Fuchs E. TGF-beta promotes heterogeneity and drug resistance in squamous cell carcinoma. *Cell* 2015; 160(5):963–76. doi: 10.1016/j.cell.2015.01.043.
18. Kasiappan R, Safe SH. ROS-inducing agents for cancer chemotherapy. *Reactive Oxygen Species* 2016; 1(1):22–37. doi: 10.20455/ros.2016.805
19. Chio, II, Jafarnejad SM, Ponz-Sarvise M, Park Y, Rivera K, Palm W, et al. NRF2 promotes tumor maintenance by modulating mrna translation in pancreatic cancer. *Cell* 2016; 166(4):963–76. doi: 10.1016/j.cell.2016.06.056.
20. Kobayashi EH, Suzuki T, Funayama R, Nagashima T, Hayashi M, Sekine H, et al. Nrf2 suppresses macrophage inflammatory response by blocking

proinflammatory cytokine transcription. *Nat Commun* 2016; 7:11624. doi: 10.1038/ncomms11624.