ROS

RESEARCH HIGHLIGHTS

ROS in Nature 2016: Part I

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ABSTRACT | Nature (along with its several sister journals) is among the most highly influential journals that publish cutting edge scientific findings in diverse fields, including biomedical sciences. The purpose of the "ROS in Nature" series is to provide a platform to introduce the high profile research discoveries related to the molecular and cell biology of reactive oxygen species (ROS) published in Nature as well as some of its sister journals, including Nature Medicine, Nature Genetics, and Nature Communications. This "ROS in Nature 2016: Part I" paper highlights the major findings relevant to ROS bioscience, and these findings are reported in four articles in Nature over the past three months (January 1, 2016–March 31, 2016). The major findings from these articles include: (1) a novel role for mitochondrial ROS in regulating thermogenesis and energy expenditure in brown and beige fat; (2) an unexpected linkage between lipid dysfunction and impaired anticancer immunosurveillance, and an essential role for mitochondria-derived ROS in this linkage; (3) autophagy as a decisive stem–cell–fate regulator and an important role for cellular (likely mitochondria-derived) ROS in this process; and finally (4) the involvement of cellular (likely mitochondria-derived) ROS in GCN2-regulated gut inflammation. The elegant studies reported in the four Nature articles attest to the continued excitement about deciphering the detailed biochemistry and cell biology of ROS, species that result from the utilization of molecular oxygen in aerobic organisms. It is hoped that introducing such high profile research discoveries on ROS would help point to the new directions for innovative research in this rapidly evolving area of bioscience.

KEYWORDS | Anticancer immunosurveillance; Autophagy; Gut inflammation; Integrated stress response; Mitochondrial ROS; Mito-Q; MitoSOX; Mito-TEMPO; Reactive oxygen species (ROS); Stem cells; Stemness; Thermogenesis; Uncoupling protein 1

ABBREVIATIONS | APC, antigen presenting cells; ATP, adenosine triphosphate; GCN2, General controlled non-repressed kinase; H_2DCFDA, 2',7'-dichlorodihydrofluorescein diacetate; ISR, integrated stress response; NAFLD, Nonalcoholic fatty liver disease; ROS, reactive oxygen species; UCP1, uncoupling protein 1

CONTENTS

1. COLD EXPOSURE–MITOCHONDRIAL ROS–UCP1–THERMOGENESIS

The term thermogenesis in biology is broadly defined as heat production resulting from the expenditure of the energy stored in food compounds and other substances in all warm-blooded animals, including humans. The total energy expenditure can be subdivided into three principal components: (1) obligatory energy expenditure required for normal physiological functioning of cells, tissues, and organs; (2) energy expenditure resulting from physical activity; and (3) energy expenditure attributed to adaptive thermogenesis [1]. Adaptive thermogenesis, also known as facultative thermogenesis, is defined operationally as heat production in response to environmental temperature or diet, and serves the purpose of protecting the organism from cold exposure or regulating energy balance after changes in diet and energy intake [1].

Brown adipose tissue, which is abundant in newborn infants and mammals (e.g., rodents) is heavily innervated by sympathetic nerves, and is responsible for a major portion of thermogenesis during cold exposure. This thermogenesis in brown as well as beige fat is regulated by uncoupling protein 1 (UCP1), a mitochondrial inner-membrane protein that uncouples proton entry from adenosine triphosphate (ATP) synthesis, thus leading to the release of heat instead of the production of ATP [2]. While UCP1 as a regulator of thermogenesis in brown and beige fat has been well recognized, the molecular basis behind cold exposure-induced UCP1-regulated thermogenic respiration remains unclear. In an article published online in March in Nature, Chouchani et al. discovered a novel role for mitochondria-derived ROS in sensitizing UCP1 to adrenergic activation [3].

They used inhibition of mitochondrial aconitase (an enzyme susceptible to superoxide-mediated inactivation via oxidative damage of the iron-sulfur cluster of the enzyme) activity as an indicator of increased mitochondrial superoxide formation and oxidation of peroxiredoxin 3 (a mitochondrial enzyme for detoxification of hydrogen peroxide) as an indicator of enhanced mitochondrial hydrogen peroxide formation. They showed that cold exposure in mice activated thermogenesis and increased the levels of mitochondrial ROS, and that inhibition of mitochondrial ROS by MitoQ (a positively charged coenzyme Q analogue that accumulates in the mitochondrial compartment) or *N*-acetylcysteine (a precursor for glutathione biosynthesis and a nonselective antioxidant compound) blunted cold exposure-induced UCP1-dependent thermogenesis [3]. Chouchani et al. went on to demonstrate that the increased levels of mitochondrial ROS caused sulfonylation of Cys253 of UCP1, leading to its increased sensitivity to adrenergic activation, thereby resulting in increased uncoupling of mitochondrial oxidative phosphorylation and consequent heat release [3]. This is the first in vivo study identifying mitochondrial ROS induction in brown adipose tissue as a novel mechanism that supports UCP1-dependent thermogenesis and whole-body energy expenditure (**Figure 1**).

While the larger array of data presented by Chouchani et al. suggested a critical role for mitochondrial ROS in mediating cold exposure-induced UCP1-dependent thermogenesis in mice in vivo, several points may deserve attention. As noted earlier, inactivation of the two mitochondrial enzymes, namely, aconitase and peroxiredoxin 3, was used to demonstrate the induction of mitochondrial ROS upon cold exposure. Mitochondrial aconitase is an iron-sulfur containing enzyme that can be oxidatively inactivated by superoxide [4]. As such, measurement of aconitase inactivation has been suggested to be used as an indirect assay for estimating mitochondrial superoxide [5]. However, this assay is not specific for superoxide as aconitase is also susceptible to other oxidant-mediated inactivation [6]. In addition, ROS-independent species such as zinc ions also inhibit mitochondrial aconitase [7]. Likewise, inactivation of mitochondrial peroxiredoxin 3 may also occur in an ROS-independent manner [8]. In fact, mitochondrial peroxiredoxin is more resilient to hyperoxidation than cytosolic isoforms of peroxiredoxins [9]. Accordingly, more definitive methods for directly measuring mitochondrial ROS would strengthen the conclusion of the study by Chouchani et al. Unfortunately, up to date, no method has been made available for direct detection of mitochondrial ROS (e.g.,

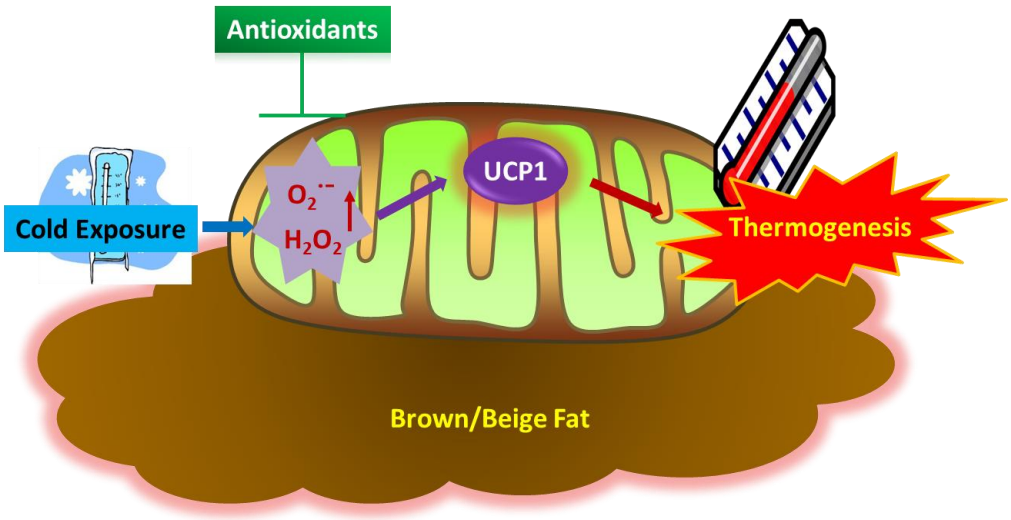

FIGURE 1. Mitochondria-derived ROS in regulating UCP1-depedent thermogenesis. $O_2^{\bullet-}$, superoxide; H_2O_2, hydrogen peroxide. See Section 1 for detailed description. This scheme is based on Ref. [3].

superoxide, hydrogen peroxide) in live animal models in vivo.

ROS is a term referring to a family of oxygen-containing reactive species, including superoxide and hydrogen peroxide, among many others. In order to understand the biology of ROS, it is important to detect the levels and identify the role of the exact members of the ROS in a physiological or pathophysiological process. In this context, it remains unknown whether mitochondria-derived superoxide or hydrogen peroxide, or a different member of ROS is directly responsible for sensitizing the activation of UCP1, causing thermogenesis. More definitive approaches could be used to investigate the involvement of either superoxide or hydrogen peroxide, or both. Transgenic overexpression of mitochondrial manganese superoxide dismutase may help delineate the role of mitochondrial superoxide in the process. In fact, a previous study suggested a possible role for superoxide to activate UCPs, including UCP1, in isolated mitochondria [10]. Recently, a selective SOD biomimetic has been developed, which can be potentially used to selectively scavenge superoxide in vivo [11]. Likewise, selective expression of catalase in mitochondria [12] can be used to determine the involvement of mitochondrial hydrogen peroxide in sensitizing UCP1 activation. In addition to the in vivo experiments, mitochondria can be isolated from the animals for determination of the changes in both superoxide and hydrogen peroxide formation using sensitive as well as definitive techniques for both superoxide and hydrogen peroxide [13–15]. Lastly, it remains unclear how cold exposure leads to increased production of mitochondrial ROS and which mitochondrial electron transport chain complexes are involved in the increased ROS release. Nevertheless, this innovative study by Chouchani et al. would yield critical insight into the physiological role of mitochondria-derived ROS in regulating thermogenesis and energy expenditure.

2. NAFLD–MITOCHONDRIAL ROS–CD4$^+$ T CELLS–HEPATOCARCINOGENESIS

Nonalcoholic fatty liver disease (NAFLD) affects a large proportion (over 10%) of the United States population and its incidence and prevalence are increasing to epidemic proportions around the world in recent decades. As with other liver diseases that cause cirrhosis, NAFLD increases the risk of hepatocellular carcinoma, a disease with poor outcomes and limited therapeutic options [16]. While cell-mediated immunity is a significant factor in influencing hepatocarcinogenesis, the detailed molecular pathways underlying NAFLD-associated liver cancer devel-

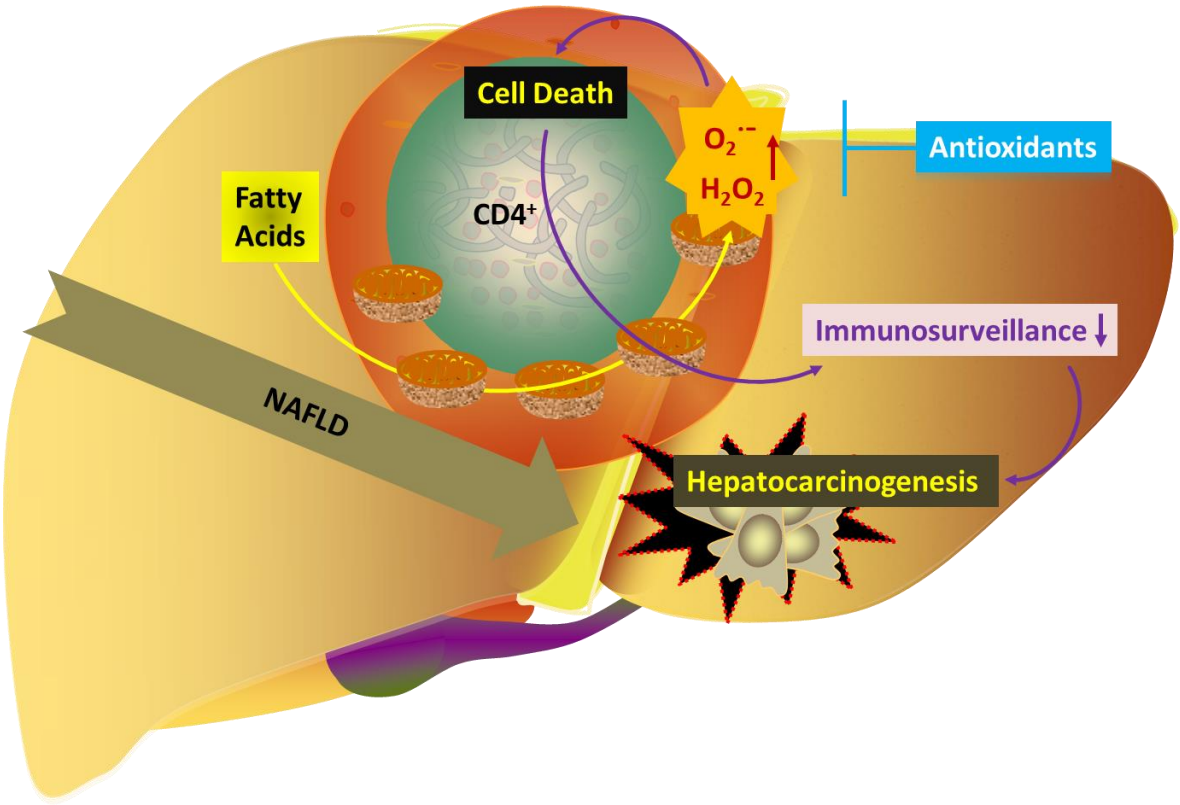

FIGURE 2. Mitochondria-derived ROS in mediating NAFLD-associated $CD4^+$ T cell loss and promotion of hepatocarcinogenesis. $O_2^{\bullet-}$, superoxide; H_2O_2, hydrogen peroxide; NAFLD, nonalcoholic fatty liver disease. See Section 2 for detailed description. This scheme is based on Ref. [17].

opment remain to be defined. In a recent Nature article [17], Ma et al., by using mouse models and human samples, demonstrated that dysregulation of lipid metabolism in NAFLD caused a selective loss of intrahepatic $CD4^+$, but not $CD8^+$, T lymphocytes, leading to accelerated hepatocarcinogenesis in mice [17]. Moreover, Ma et al. observed that $CD4^+$ T lymphocytes possessed greater mitochondrial mass than $CD8^+$ T lymphocytes and generated higher levels of mitochondria-derived ROS.

Ma and coworkers employed the method of MitoSOX-based flow cytometry to detect mitochondrial ROS in the T lymphocytes. MitoSOX is often cited as a selective probe for mitochondrial superoxide, but the validity of this claim has been questioned [18], and the probe at the concentrations typically used also causes mitochondrial uncoupling and inhibition of complex IV [19].

Ma et al. further showed that disruption of mitochondrial function by linoleic acid, a fatty acid accumulated in NAFLD, elicited more oxidative damage than other free fatty acids (e.g., palmitic acid), and induced selective loss of intrahepatic $CD4^+$ T lymphocytes [17]. Regardless of the potential concerns on the validity of using MitoSOX as well as other fluorescence probes, such as the most commonly used 2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA) for detecting cellular ROS, treatment with Mito-TEMPO (an antioxidant compound that accumulates in mitochondria due to its positive charge) or *N*-acetylcysteine (a glutathione precursor and non-selective antioxidant) inhibited fatty acid-induced ROS formation as well as $CD4^+$ cell death [17]. Importantly, the study by Ma et al. demonstrated that in vivo blockade of ROS by *N*-acetylcysteine or Mito-TEMPO reversed NAFLD-

induced hepatic $CD4^+$ T lymphocyte death and delayed NAFLD-promoted hepatocarcinogenesis in mice [17]. This elegant study by Ma et al. [17] suggested a possible causal involvement for mitochondria-derived ROS in the selective loss of intrahepatic $CD4^+$ T lymphocytes and the consequent promotion of hepatocarcinogenesis in NAFLD (**Figure 2**).

It has been established that mitochondrial ROS play important roles under both physiological and pathophysiological conditions [20]. In this context, multiple recent studies have demonstrated that the tightly regulated formation of mitochondrial ROS serves as an important signaling mechanism for T cell immunity as well as innate immunity [21–23], and as such, inhibition of this regulated formation of mitochondrial ROS by antioxidant compounds would compromise T cell immunity, leading to detrimental effects. On the other hand, dysregulated induction of mitochondrial ROS, as suggested by the study of Ma et al. [17] in NAFLD, may cause selective $CD4^+$ T cell depletion, leading to compromised immunosurveillance and consequent promotion of hepatocarcinogenesis. Under this condition, antioxidants would help protect against intrahepatic $CD4^+$ T cell death and thereby inhibit hepatocarcinogenesis in NAFLD. Hence, mitochondrial ROS (as well as cellular ROS in general) can be a double-edged sword causing benefit and doing harm under different conditions. Accordingly, antioxidant therapy should be tailored to protect against the harm caused by ROS and preserve the beneficial effects of these oxygen-containing reactive species.

3. AUTOPHAGY–ROS–STEM CELL FATE

Autophagy involves the sequestration of cytoplasmic components, which can be entire organelles, lipid vesicles, or protein aggregates, within a double-membraned vesicle, known as autophagosome. Autophagosomes fuse with lysosomes to generate autolysosomes, in which the autophagic cargo is degraded by acidic hydrolases. Autophagy is an evolutionarily conserved mechanism of adaptation to stressful microenvironmental conditions, including limited nutrient supplies [24]. Autophagy preserves the health of cells and tissues by replacing obsolete and damaged cellular components such as dysfunctioning mitochondria with newly made ones. Damaged and dysfunctioning mitochondria release more ROS. Likewise, as noted above, under starvation, autophagy provides an internal source of nutrients for energy generation, thereby maintaining the survival of the cells and tissues.

Autophagy, as a powerful promoter of metabolic homeostasis, prevents degenerative diseases; however, cancer cells also exploit this mechanism to survive in nutrient-poor tumors [25]. Hence, autophagy, like ROS, might be viewed as a double-edged sword and plays different roles under different conditions.

The study by García-Prat et al. reported in the January 7 issue of Nature described a novel role for autophagy in maintaining the fate of stem cells via preventing senescence induced by ROS [26]. This novel study by García-Prat et al. showed that in adult resting muscle, quiescent stem cells attenuated proteotoxicity by maintaining a high basal autophagy flux, constituting a homeostatic 'clean up' process [26]. This 'clean up' function is considered critical in non-dividing stem cells, in which mitotic dilution of intracellular toxic debris does not take place. The study by García-Prat et al. [26] pointed out that autophagy failure in aged resting stem cells led to accumulation of damaged proteins and dysfunctional organelles, specially mitochondria, which produced elevated ROS levels that caused DNA damage and senescence entry. Indeed, elevated levels of mitochondrial ROS play an important role in the induction of senescence [27, 28]. Hence, normal function of autophagy helps clean up the damaged mitochondria that would release more ROS, thereby preventing mitochondrial ROS-induced senescence of stem cells (**Figure 3**).

It is also worth mentioning that in certain models, such as *C. elegans*, mitochondrial ROS are instead involved in the prolongation of the longevity of the organism. It is believed that in this organism, mitochondrial ROS elicit protective mechanisms that keep the organism alive under stressful conditions [29]. Likewise, ROS generated by NOX1 (NADPH oxidase 1) play a critical role in mouse spermatogonial stem cell self-renewal via the activation of the p38 MAPK and JNK pathways [30]. A more recent study suggested that transient activation of mitochondrial ROS at the early stage enhanced cell reprogramming, whereas sustained activation impaired cell reprogramming [31]. Hence, the source and location, the rate, and the duration of ROS production, as well as the type of animal species involved all impact the role of ROS in cell fate determination.

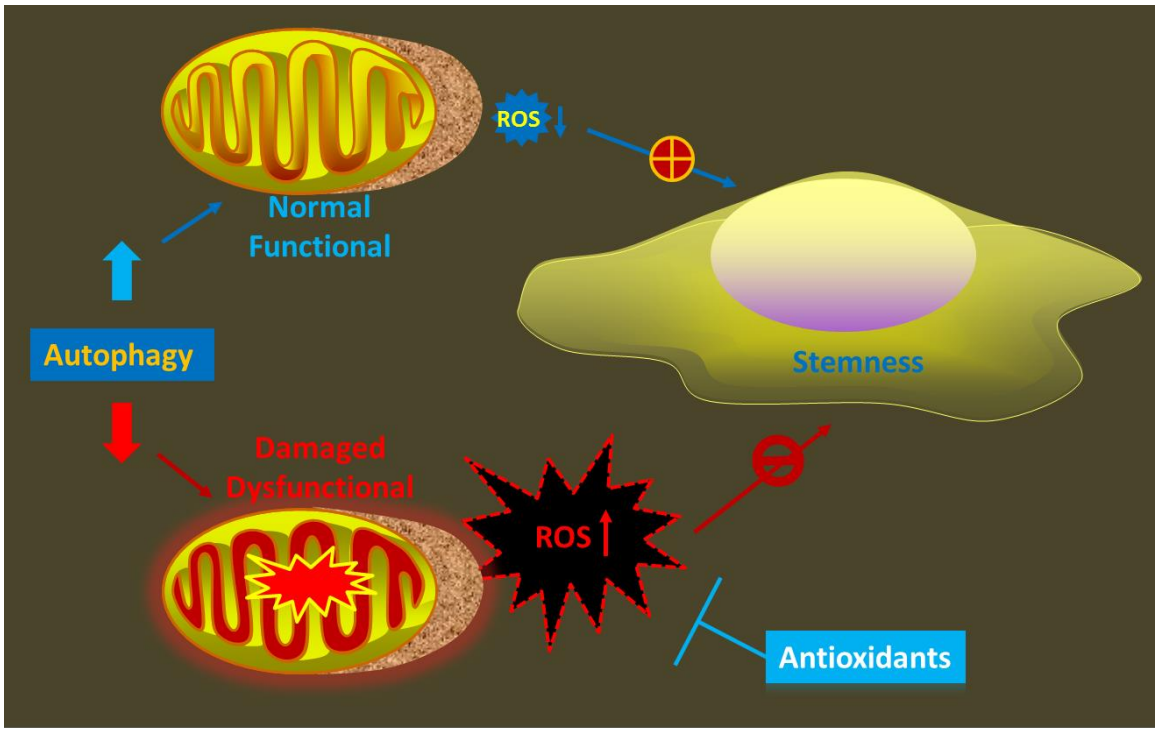

FIGURE 3. Involvement of cellular (possibly mitochondria-derived) ROS in autophagy regulation of stem cell fate. See Section 3 for detailed description. This scheme is based on Ref. [26].

4. GCN2–AUTOPHAGY–ROS–GUT INFLAMMATION

As indicated earlier, during the response to sublethal stress such as starvation or oxidative stress, cells undergo rapid changes to adapt their metabolism and protect themselves against potential damage. This is orchestrated through a multifaceted cellular program, which involves the concerted action of diverse stress response pathways, and hence the term 'integrated stress response' (IRS) has been coined to describe this phenomenon. ISR can be defined literally as a homeostatic mechanism by which eukaryotic cells sense and respond to stress-inducing signals, such as amino acid starvation and oxidative stress [32, 33]. In fact, autophagy described above is part of the ISR. Besides autophagy, the cellular response to stress involves numerous other pathways including those that regulate nutrient uptake, intermediary metabolism, cell cycle and growth control, cell fate and lineage decisions, antioxidant defenses, and cellular survival/death programs. Therefore, it is not surprising that there is a close integration between signals that regulate these cellular processes [33].

General controlled non-repressed (GCN2) kinase, a serine/threonine-protein kinase, is a key orchestrator of the ISR, and modulates protein synthesis in response to amino acid starvation. In a recent Nature article [34], Ravindran et al. reported that GCN2 controlled intestinal inflammation by suppressing inflammasome activation in mice. Enhanced activation of ISR was observed in intestinal antigen presenting cells (APCs) and epithelial cells during amino acid starvation, or intestinal inflammation [34]. Genetic deletion of GCN2 in the above cell types resulted in enhanced intestinal inflammation owing to enhanced inflammasome activation and interleukin-1β production [34]. Importantly, they found that this enhanced gut inflammation was caused by reduced autophagy in GCN2-deficient intestinal APCs and epithelial cells, leading to increased formation ROS (as detected by H_2DCFDA and MitoSOX assays), which are known to act as potent activators of inflammasomes [34]. Notably, in vivo

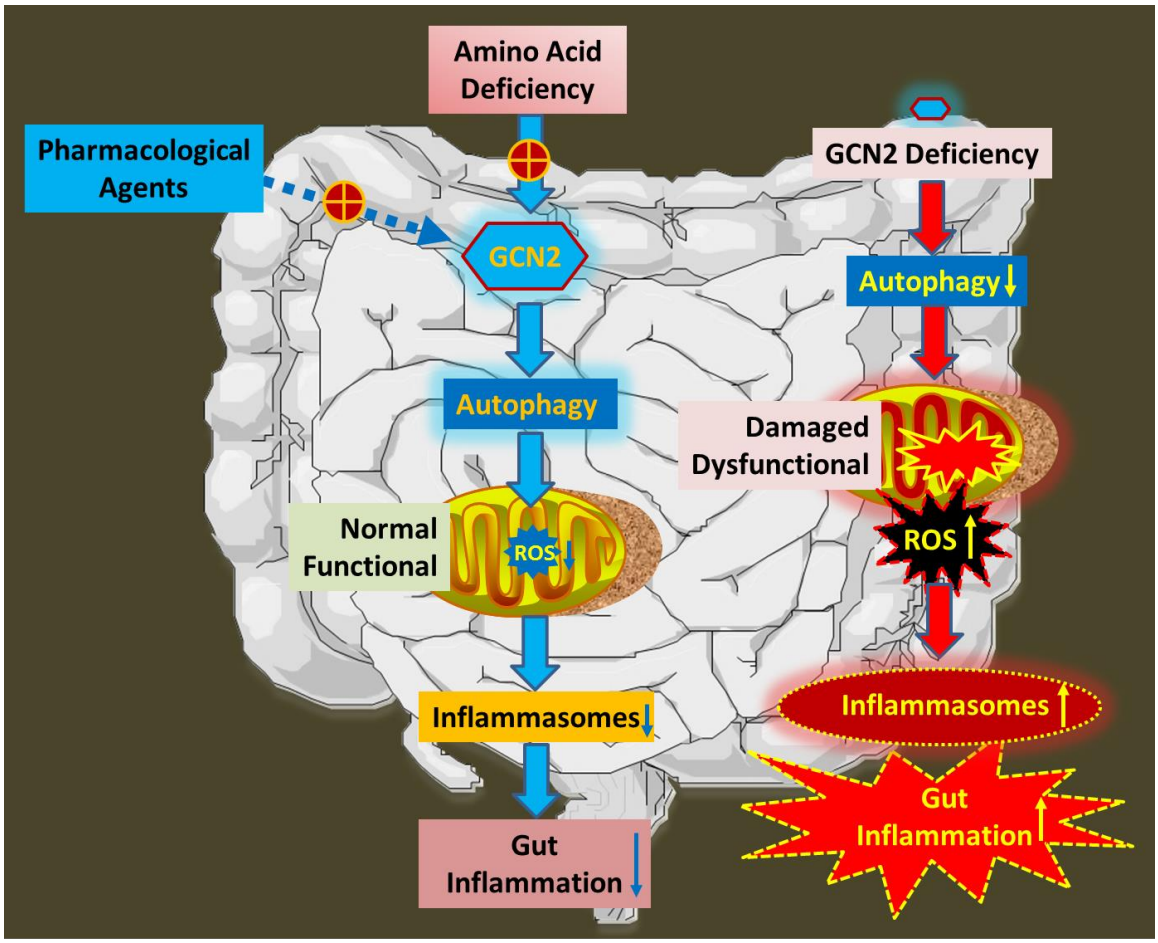

FIGURE 4. Involvement of cellular (possibly mitochondria-derived) ROS in GCN2/autophagy inhibition of gut inflammation. See Section 4 for detailed description. This scheme is based on Ref. [36].

blockade of ROS by *N*-acetylcysteine (a glutathione precursor and a nonselective antioxidant) resulted in inhibition of gut inflammation in GCN2-null mice [34]. Lastly, the study demonstrated that acute amino acid starvation suppressed intestinal inflammation via a mechanism dependent on GCN2 [34]. Hence, it appeared that the 'GCN2–autophagy–ROS (mitochondrial ROS)' axis played an important part in modulating gut inflammation (**Figure 4**).

This exciting study by Ravindran et al. provides another example showing a potential physiological function of autophagy in suppressing cellular/mitochondrial ROS formation and thereby inhibiting inflammatory stress. This study would likely give an impetus to the continued research aiming to decipher the biological function of GCN2, a key orchestrator of the ISR, in controlling oxidative and inflammatory stress underlying not only gut disorders, but also other common diseases, such as neurological and cardiovascular disorders. Research in this area may lead to the development of pharmacological modalities that can be used to stimulate GCN2 for the intervention of oxidative stress and inflammatory disorders. In this context, it would be also important to determine the potential role of GCN2 in regulating cellular antioxidant defenses. In this context, a recent study suggested a possible involvement for GCN2 in regulating the increased synthesis of glutathione, a major cellular antioxidant, under stress conditions [35].

5. CONCLUDING REMARKS

The four Nature articles introduced in this "ROS in Nature 2016: Part I" paper present the scientific communities with four major discoveries in the science of ROS during the first quarter of 2016. These cutting edge findings are: (1) mitochondrial ROS as a regulator of UCP1-dependent thermogenesis; (2) mitochondrial ROS as a cause of intrahepatic loss of $CD4^+$ T cells, thereby promoting hepatocarcinogenesis in NAFLD; (3) increased cellular (possibly mitochondria-derived) ROS as a mediator of stem cell senescence due to compromised autophagy; and finally (4) suppression of cellular ROS (possibly mitochondrial ROS) as a potential mechanism underlying GCN2-mediated inhibition of gut inflammation. Collectively, the above novel findings on ROS reported in Nature would further advance our current knowledge on ROS in biology and medicine and open up new avenues of innovative ROS research.

REFERENCES

1. Lowell BB, Spiegelman BM. Towards a molecular understanding of adaptive thermogenesis. *Nature* 2000; 404(6778):652–60. doi: 10.1038/35007527.
2. Fedorenko A, Lishko PV, Kirichok Y. Mechanism of fatty-acid-dependent UCP1 uncoupling in brown fat mitochondria. *Cell* 2012; 151(2):400–13. doi: 10.1016/j.cell.2012.09.010.
3. Chouchani ET, Kazak L, Jedrychowski MP, Lu GZ, Erickson BK, Szpyt J, et al. Mitochondrial ROS regulate thermogenic energy expenditure and sulfenylation of UCP1. *Nature* 2016 (in press). doi: 10.1038/nature17399.
4. Hopkins RZ. Superoxide in biology and medicine: an overview. 2016; 1(2):99-109. doi: 10.20455/ros.2016.825.
5. Fridovich I. Superoxide anion radical ($O_2^{\cdot-}$), superoxide dismutases, and related matters. *J Biol Chem* 1997; 272(30):18515–7.
6. Tarpey MM, Fridovich I. Methods of detection of vascular reactive species: nitric oxide, superoxide, hydrogen peroxide, and peroxynitrite. *Circ Res* 2001; 89(3):224–36.
7. Costello LC, Liu Y, Franklin RB, Kennedy MC. Zinc inhibition of mitochondrial aconitase and its importance in citrate metabolism of prostate epithelial cells. *J Biol Chem* 1997; 272(46):28875–81.
8. Radi R. Peroxynitrite, a stealthy biological oxidant. *J Biol Chem* 2013; 288(37):26464–72. doi: 10.1074/jbc.R113.472936.
9. Cox AG, Pearson AG, Pullar JM, Jonsson TJ, Lowther WT, Winterbourn CC, et al. Mitochondrial peroxiredoxin 3 is more resilient to hyperoxidation than cytoplasmic peroxiredoxins. *Biochem J* 2009; 421(1):51–8. doi: 10.1042/BJ20090242.
10. Echtay KS, Roussel D, St-Pierre J, Jekabsons MB, Cadenas S, Stuart JA, et al. Superoxide activates mitochondrial uncoupling proteins. *Nature* 2002; 415(6867):96–9. doi: 10.1038/415096a.
11. Bird HZ, Hopkins RZ. Nanomaterials for selective superoxide dismutation. *Reactive Oxygen Species* 2016; 1(1):59–64. doi: 10.20455/ros.2016.811.
12. Schriner SE, Linford NJ, Martin GM, Treuting P, Ogburn CE, Emond M, et al. Extension of murine life span by overexpression of catalase targeted to mitochondria. *Science* 2005; 308(5730):1909–11. doi: 10.1126/science.1106653.
13. Zhu H. Assays for detecting biological superoxide. *Reactive Oxygen Species* 2016; 1(1):65–80. doi: 10.20455/ros.2016.813.
14. Zhu H, Jia Z, Trush MA, Li YR. A Highly sensitive chemiluminometric assay for real-time detection of biological hydrogen peroxide formation. *Reactive Oxygen Species* 2016; 1(3):216-227. doi: 10.20455/ros.2016.844
15. Ermakova YG, Bilan DS, Matlashov ME, Mishina NM, Markvicheva KN, Subach OM, et al. Red fluorescent genetically encoded indicator for intracellular hydrogen peroxide. *Nat Commun* 2014; 5:5222. doi: 10.1038/ncomms6222.
16. Michelotti GA, Machado MV, Diehl AM. NAFLD, NASH and liver cancer. *Nat Rev Gastroenterol Hepatol* 2013; 10(11):656–65. doi: 10.1038/nrgastro.2013.183.
17. Ma C, Kesarwala AH, Eggert T, Medina-Echeverz J, Kleiner DE, Jin P, et al. NAFLD causes selective $CD4^+$ T lymphocyte loss and promotes hepatocarcinogenesis. *Nature* 2016; 531(7593):253–7. doi: 10.1038/nature16969.
18. Kalyanaraman B, Darley-Usmar V, Davies KJ, Dennery PA, Forman HJ, Grisham MB, et al. Measuring reactive oxygen and nitrogen species with fluorescent probes: challenges and limitations. *Free Radic Biol Med* 2012; 52(1):1–6. doi: 10.1016/j.freeradbiomed.2011.09.030.
19. Roelofs BA, Ge SX, Studlack PE, Polster BM. Low micromolar concentrations of the superoxide probe MitoSOX uncouple neural mitochondria and

inhibit complex IV. *Free Radic Biol Med* 2015; 86:250–8. doi: 10.1016/j.freeradbiomed.2015.05.032.
20. Shadel GS, Horvath TL. Mitochondrial ROS signaling in organismal homeostasis. *Cell* 2015; 163(3):560–9. doi: 10.1016/j.cell.2015.10.001.
21. Sena LA, Li S, Jairaman A, Prakriya M, Ezponda T, Hildeman DA, et al. Mitochondria are required for antigen-specific T cell activation through reactive oxygen species signaling. *Immunity* 2013; 38(2):225–36. doi: 10.1016/j.immuni.2012.10.020.
22. West AP, Brodsky IE, Rahner C, Woo DK, Erdjument-Bromage H, Tempst P, et al. TLR signalling augments macrophage bactericidal activity through mitochondrial ROS. *Nature* 2011; 472(7344):476–80. doi: 10.1038/nature09973.
23. Okoye I, Wang L, Pallmer K, Richter K, Ichimura T, Haas R, et al. T cell metabolism. The protein LEM promotes $CD8^+$ T cell immunity through effects on mitochondrial respiration. *Science* 2015; 348(6238):995–1001. doi: 10.1126/science.aaa7516.
24. Galluzzi L, Pietrocola F, Levine B, Kroemer G. Metabolic control of autophagy. *Cell* 2014; 159(6):1263–76. doi: 10.1016/j.cell.2014.11.006.
25. Rabinowitz JD, White E. Autophagy and metabolism. *Science* 2010; 330(6009):1344–8. doi: 10.1126/science.1193497.
26. Garcia-Prat L, Martinez-Vicente M, Perdiguero E, Ortet L, Rodriguez-Ubreva J, Rebollo E, et al. Autophagy maintains stemness by preventing senescence. *Nature* 2016; 529(7584):37–42. doi: 10.1038/nature16187.
27. Holmstrom KM, Finkel T. Cellular mechanisms and physiological consequences of redox-dependent signalling. *Nat Rev Mol Cell Biol* 2014; 15(6):411–21. doi: 10.1038/nrm3801.
28. Balaban RS, Nemoto S, Finkel T. Mitochondria, oxidants, and aging. *Cell* 2005; 120(4):483–95. doi: 10.1016/j.cell.2005.02.001.
29. Yee C, Yang W, Hekimi S. The intrinsic apoptosis pathway mediates the pro-longevity response to mitochondrial ROS in *C. elegans*. *Cell* 2014; 157(4):897–909. doi: 10.1016/j.cell.2014.02.055.
30. Morimoto H, Iwata K, Ogonuki N, Inoue K, Atsuo O, Kanatsu-Shinohara M, et al. ROS are required for mouse spermatogonial stem cell self-renewal. *Cell Stem Cell* 2013; 12(6):774–86. doi: 10.1016/j.stem.2013.04.001.
31. Ying Z, Chen K, Zheng L, Wu Y, Li L, Wang R, et al. Transient activation of mitoflashes modulates nanog at the early phase of somatic cell reprogramming. *Cell Metab* 2016; 23(1):220–6. doi: 10.1016/j.cmet.2015.10.002.
32. Harding HP, Zhang Y, Zeng H, Novoa I, Lu PD, Calfon M, et al. An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. *Mol Cell* 2003; 11(3):619–33.
33. Kroemer G, Marino G, Levine B. Autophagy and the integrated stress response. *Mol Cell* 2010; 40(2):280–93. doi: 10.1016/j.molcel.2010.09.023.
34. Ravindran R, Loebbermann J, Nakaya HI, Khan N, Ma H, Gama L, et al. The amino acid sensor GCN2 controls gut inflammation by inhibiting inflammasome activation. *Nature* 2016; 531(7595):523–7. doi: 10.1038/nature17186.
35. Sikalidis AK, Mazor KM, Lee JI, Roman HB, Hirschberger LL, Stipanuk MH. Upregulation of capacity for glutathione synthesis in response to amino acid deprivation: regulation of glutamate-cysteine ligase subunits. *Amino Acids* 2014; 46(5):1285–96. doi: 10.1007/s00726-014-1687-1.