ROS

RESEARCH HIGHLIGHTS

ROS in Cell 2015

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ABSTRACT | The journal Cell is among the most influential journals in the field of bioscience. The purpose of the "ROS in Cell" series is to provide a platform for introducing and discussing cutting edge scientific findings on ROS bioscience reported in Cell. This is to foster critical thinking on future directions of innovative research on ROS and the potential translation of the leading edge knowledge on ROS in biology and medicine. Accordingly, this ROS in Cell 2015 paper describes four major research articles on ROS, which were published in Cell in 2015. The major findings reported in these articles include: (1) peroxisome as an anti-oxidative organelle in protecting against hearing loss; (2) the anti-oxidative and pro-oxidative roles of lipid droplets in stem cell biology as well as neurodegeneration; and (3) a novel role for the 'eIF4E–antioxidants' axis in oncogenic transformation and tumorigenesis. These exciting discoveries contribute greatly to our current understanding of the molecular science of ROS and open up new directions for future innovative basic and translational research on ROS in biology and medicine.

KEYWORDS | Antioxidants; Eukaryotic translation initiation factor 4E; Hearing loss; Lipid droplets; Neurodegeneration; Noise; Oxidative stress; Perjvakin; Peroxisome; Stem cell

ABBREVIATIONS | eIF4E, eukaryotic translation initiation factor 4E; JNK, c-Jun-N-terminal kinase; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species; SREBP, sterol regulatory element binding protein

CONTENTS

1. PEROXISOME AS AN ANTI-OXIDATIVE ORGANELLE

Peroxisomes are single-membrane subcellular organelles, found in virtually all eukaryotic cells and were originally defined as organelles that contain at least one oxidase and catalase for the respective production and decomposition of hydrogen peroxide [1]. Peroxisome biogenesis involves two pathways: (1) the de novo pathway, whereby preperoxisomal vesi-

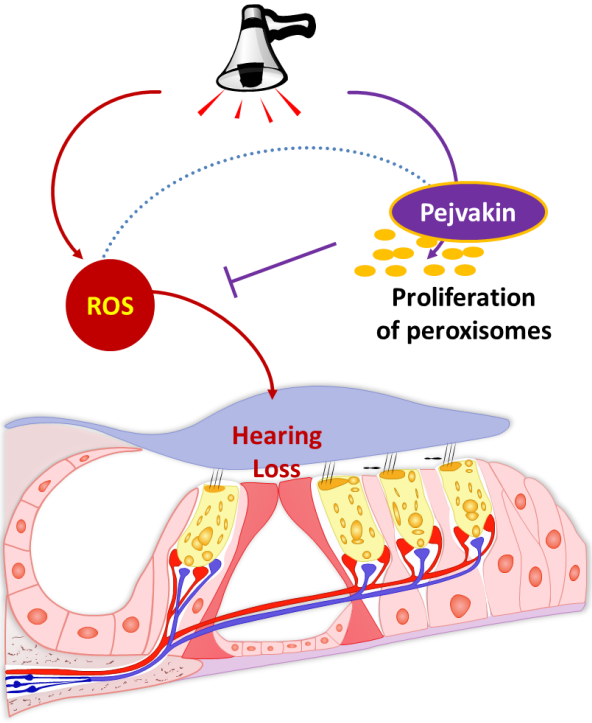

FIGURE 1. Peroxisome as a potentially novel anti-oxidative organelle in protecting against hearing loss. See text (Section 1) for detailed discussion. This scheme is based on Ref. [6].

cles bud from the endoplasmic reticulum and subsequently fuse with each other to form mature peroxisomes; and (2) fission, whereby new peroxisomes result from the growth and division (fission) of pre-existing peroxisomes [2]. Depending on the cell type and the environment, peroxisomes perform diverse tightly regulated functions, the most notable of which are linked to lipid metabolism, especially the breakdown of long chain fatty acids via beta-oxidation into medium chain fatty acids that subsequently enter mitochondria for further oxidation.

The functions of peroxisomes are integrated with processes in other cellular compartments, including mitochondria, lysosomes, and the cytosol, as well as lipid droplets through the existence of both shared and coordinated metabolic pathways [2, 3]. In this context, defects and dysfunctions of peroxisomes are involved in diverse conditions (collectively known as peroxisomal disorders), including those that affect the central nervous systems [2]. Recent studies suggest that peroxisomes are not merely metabolic organelles, but also act as signaling platforms for promoting antiviral immunity and other physiological processes [4, 5]. These novel findings unmask the new faces of peroxisomes in cell physiology and pathophysiology.

In an article published in the November 5, 2015 issue of Cell, Delmaghani et al. reported a novel antioxidant role for peroxisomes in protecting the auditory system from noise-induced damage [6], which further expands the spectrum of peroxosomal functions. In their elegant study, Delmaghani et al. employed the pejvakin-deficient ($Pjvk^{-/-}$) mice as an experimental model of hearing loss. Pejvakin, a 352-residue protein with unknown function is encoded by a newly identified gene DFNB59, whose mutation is implicated in auditory neuropathy and hearing loss [7]. Pejvakin is a paralog of DFNA5, a protein of unknown function that is also involved in deafness [7]. Using $Pjvk^{-/-}$ mice, Delmaghani et al. showed that pejvakin deficiency resulted in hypervulnerability to the natural acoustic environment, and the absence of pejvakin affected the propagation of action potentials in the auditory pathway after both controlled electrical and sound exposure [6]. Notably, pejvakin deficiency caused decreased antioxidant defenses and increased oxidative stress in cochlea, impacting various electrophysiological properties of the hair cells, particularly the mechanoelectrical transduction and K^+ current through voltage- and Ca^{2+}-activated potassium channels [6].

Delmaghani et al. went on to further show that pejvakin was a peroxisome-associated protein involved in oxidative stress- and sound-induced peroxisome proliferation in hair cells as well as auditory neurons [6]. It is worth mentioning that oxidative stress is an important mechanism of noise-induced hearing impairment [8, 9]. Deficiency of pejvakin resulted in the failure of peroxisome proliferation upon sound exposure, and this impaired adaptive response could be partially restored by pejvakin re-expression in the $Pjvk^{-/-}$ mice [6]. In view of the oxidative stress mechanism of sound-induced hearing impartment, Delmaghani et al. examined the therapeutic effects of antioxidant compounds, and found that treating $Pjvk^{-/-}$ mice with *N*-acetylcysteine could provide a small, but significant improvement of hearing [6].

N-Acetylcysteine is a glutathione precursor and non-selective antioxidant. Although it is widely used as an antioxidant compound, *N*-acetylcysteine is in

fact a relatively weak antioxidant, and as such, millimolar concentrations are frequently needed to provide protection in experimental models. In this concontext, it is imperative to investigate the potential therapeutic effects of other more effective antioxidants (such as superoxide dismutase/catalase mimetics or antioxidant enzyme gene therapy) in sound-induced hearing impairment in Pjvk$^{-/-}$ mice.

Peroxisomes house diverse ROS-producing enzymes and are also equipped with antioxidant defenses, especially catalase, making the organelles functioning as both a source and a sink of ROS [10]. Although the proliferation of peroxisomes was previously shown to be associated with decreased cellular ROS under certain conditions [11], the exact molecular basis behind the reduction of ROS remains unclear. Nevertheless, the study by Delmaghani et al. [6] demonstrated that noise exposure rapidly upregulated Pjvk cochlear transcription in wild-type mice and triggered peroxisome proliferation in hair cells and primary auditory neurons, and suggested a potential antioxidant activity of peroxisomes in protecting the auditory system against noise-induced oxidative stress and hearing damage (**Figure 1**).

2. LIPID DROPLETS: DANCING BETWEEN ANTI-OXIDATION AND PRO-OXIDATION

Long perceived as inert fat particles, lipid droplets had been largely ignored by cell biologists until a few years ago. More recently, lipid droplets are increasingly recognized as dynamic organelles that fulfil important functions [12–14]. These ubiquitous organelles are found in most eukaryotic cells. They range greatly in size with diameter being anywhere from less than 1 μm to 100 μm, and each droplet consists of a phospholipid monolayer that surrounds a core of neutral lipids, such as sterol esters or triglycerides. Numerous proteins, many of which play functional roles in lipid droplet biology, decorate surfaces of the particles. Lipid droplets are therefore structurally similar to plasma lipoprotein particles, which are secreted from cells and transport lipids through the aqueous circulation to different sites of the body. We are just beginning to understand fundamental aspects of lipid droplet biogenesis, catabolism, and functional activities in cells [12–14].

Lipid droplets are best known as the intracellular sites for neutral lipid storage. These droplets are in fact critical for lipid metabolism and energy homeostasis, and their dysfunction has been linked to many diseases, including obesity and cardiovascular disorders [15, 16]. Accumulating evidence suggests that the roles that lipid droplets play in biology are significantly broader than previously anticipated. For instance, lipid droplets are the source of molecules important in the nucleus: they can sequester transcription factors and chromatin components and generate the lipid ligands for certain nuclear receptors, thereby impacting cell signal transduction. Lipid droplets have also emerged as important nodes for fatty acid trafficking, both inside the cell and between cells [17]. Another emerging function for lipid droplets is the coordination of immune responses, as these organelles participate in the generation of prostaglandins and leukotrienes, which are important inflammation mediators. Lipid droplets may also play a part in interferon responses and in antigen cross presentation. Many pathogens, including hepatitis C and Dengue viruses, *Chlamydia*, and *Mycobacterium*, may target lipid droplets during infection either for nutritional purposes or as part of an anti-immunity strategy [15, 18].

In two recent articles published in Cell in 2015 [19, 20], lipid droplets were found to play an important role in neuronal stem cell biology and neurodegeneration via serving as a potential antioxidant defense and acting as a source of lipid peroxidation, respectively, highlighting the increasing complexity of these unique organelles in cell biology and pathophysiology. In the first article, Bailey et al., by studying *Drosophila* larvae development, demonstrated that glial cell niche (a specialized microenvironment where neural stem cells reside) preserved the proliferation of neural stem cells/neuroblasts under conditions of hypoxia and oxidative stress [19]. Hypoxia is known to stimulate ROS generation and provoke oxidative stress [21]. Notably, Bailey et al. found that hypoxia and oxidative stress resulted in increased accumulation of lipid droplets, and the lipid droplets that formed in niche glia during oxidative stress limited the levels of ROS and suppressed the peroxidation of polyunsaturated fatty acids (PUFAs) [19]. PUFAs, due to their rich C=C double bonds, are particularly susceptible to ROS-induced peroxidation [22]. Bailey et al. further found that the lipid droplets protected glia and neuroblasts from PUFA peroxidation, thereby maintaining neuroblast proliferation under hypoxia/oxidative stress conditions. Important-

ly, Bailey et al. went on to demonstrate that oxidative stress caused the redistribution of PUFAs from the outer phospholipid layer and cell membranes to the triglyceride core of the droplets, thereby shielding these ROS-susceptible lipids from undergoing peroxidation reaction to form secondary toxic species, including reactive aldehydes that can cause damage to key cellular biomolecules [19]. Accordingly, Bailey et al. proposed an antioxidant function for lipid droplets via the above mechanism in protecting neuronal stem cell proliferation during *Drosophila* larvae development. As lipid droplets are ubiquitously present in most cell types, the antioxidant function of these organelles warrants further investigation in other systems and under various conditions. In this context, the second article by Liu et al. published in the January 15, 2015 issue of Cell suggested that lipid droplets could behave in a pro-oxidative manner to contribute to neurodegeneration in adult *Drosophila* as well as mice [20].

It has been extensively demonstrated that ROS and mitochondrial defects are intimately intertwined events that play a critical role in the development of neurodegeneration [23, 24]. Using adult *Drosophila* with mitochondrial defects as an experimental model for neurodegeneration, Liu et al. recently reported that a key consequence of oxidative stress and neuronal mitochondrial dysfunction was the accumulation of lipid droplets in glia [20]. This is in line with the observation made by Bailey et al in *Drosophila* larvae [19], as discussed above, i.e., ROS and oxidative stress stimulated lipid droplet accumulation. The mechanistic studies by Liu et al. revealed that ROS triggered c-Jun-N-terminal kinase (JNK) and sterol regulatory element binding protein (SREBP) activity in neurons of adult *Drosophila*, leading to lipid droplet accumulation in glia prior to or at the onset of neurodegeneration [20]. Hence, the study by Liu et al. elucidated an important role for the JNK/SREBP signaling in oxidative stress-induced lipid droplet accumulation in glia. Conversely, suppression of JNK/SREBP signaling may thus be a valid approach to attenuating ROS-induced lipid droplet accumulation and the resulting pathophysiological conditions [20]. In this context, Liu et al. further showed that the accumulated lipids/lipid droplets underwent lipid peroxidation in the presence of ROS. Notably, they demonstrated that decreasing lipid droplet accumulation in glia and lipid peroxidation via targeted lipase overexpression and/or lowering ROS (via genetic overexpression of Cu, Zn superoxide dismutase or using the antioxidant compound *N*-acetylcysteine amide) significantly delayed the onset of neurodegeneration [20]. *N*-Acetylcysteine amide is a membrane permeable analog of *N*-acetylcysteine, and this amide derivative can cross the blood-brain barrier and provide neuroprotection under various conditions [25, 26]. Importantly, by using a mitochondrial complex I subunit Ndufs4-knockout mouse model, Liu et al. elegantly demonstrated a similar pathway leading to glial lipid droplet accumulation in Ndufs4 mutant mice with neuronal mitochondrial defects, as well as the delayed onset of neurodegeneration and motor defects by treatment of the Ndufs4-deficient mice with *N*-acetylcysteine amide [20]. It is worth mentioning that a key finding of the study by Liu et al. was that the mere accumulation of lipid droplets was not sufficient to promote the neurodegeneration process, and the concomitant presence of ROS (derived from mitochondrial defects) was required. In the presence of ROS the accumulated lipids/lipid droplets underwent lipid peroxidation to generate reactive species that promote neurodegeneration. Hence, a synergistic mechanism between lipid droplet accumulation and concomitant ROS presence appeared to operate to contribute to neurodegeneration in the *Drosophila* as well as the mouse model.

The apparently conflicting conclusions about the anti-oxidative and pro-oxidative properties of lipid droplets from the two Cell articles highlight the complexity of lipid droplet biology with regard to the involvement of these unique organelles in neurophysiology and neuropathophysiology (**Figure 2**). The seemingly opposite effects of lipid droplets might be explained by differences in ROS levels or the intensity of oxidative stress in the cellular environment. Relatively low levels of ROS (as seen during development of *Drosophila* larvae) cause accumulation of lipid droplets as an adaptive response to protect against mild oxidative stress injury because of the ability of lipid droplets to help shield PUFAs from ROS-mediated lipid peroxidation. On the other hand, relatively low or moderate levels of ROS (as seen in adult animals and during aging) cause accumulation of lipid droplets, and such increased lipid droplets would elevate the total mass of PUFAs (though some will distribute to the core of the droplets). In the continued presence of ROS, especially when the levels of ROS are markedly ele-

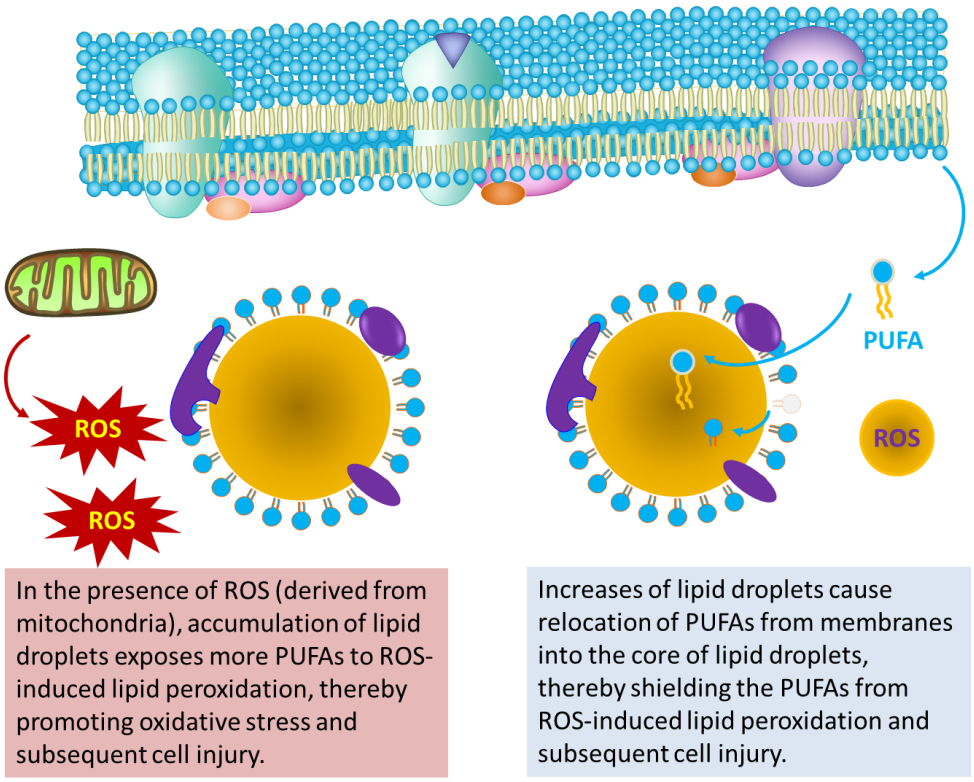

FIGURE 2. Dancing of lipid droplets between the stages of anti-oxidation and pro-oxidation. See text (Section 2) for detailed discussion. This scheme is based on Refs. [19, 20].

vated (as seen in adult or aging animals with mitochondrial defects), the reaction between ROS and the increased total mass of PUFAs would result in markedly increased lipid peroxidation, leading to the formation of secondary reactive species (e.g., aldehydes) to promote oxidative cell injury. Hence, the cellular environment and ROS levels might determine on which stage lipid droplets would dance. The ability of lipid droplets to dance between the anti-oxidation and pro-oxidation stages under different conditions necessitates that particular attention should be paid when designing strategies for disease intervention by targeting these organelles.

3. THE 'EIF4E–ANTIOXIDANTS' AXIS IN ONCOGENIC TRANSFORMATION

Translational control of gene expression plays a key role in many biological processes. Consequently, the activity of the translation apparatus is under tight homeostatic control. Eukaryotic translation initiation factor 4E (eIF4E), the mRNA 5' cap-binding protein, facilitates cap-dependent translation and is a major target for translational control [27]. While eIF4E is crucial for normal growth, increased activity of eIF4E is involved in tumorigenesis. eIF4E controls gene expression through its effects on cap-dependent translation as well as mRNA export, both of which contribute to its oncogenic potential [28]. The important role of eIF4E in cell homeostasis is also reflected by its regulation at different levels (e.g., the presence of endogenous proteins that inhibit the functional activity of eIF4E [29, 30].

In the July 2, 2015 issue of Cell, Truitt et al. reported an exciting finding linking eIF4E gene dosage to cellular antioxidants-ROS homeostasis and tumorigenesis, thereby highlighting a novel mechanism by which eIF4E drives tumorigenesis via modulating cellular antioxidants/ROS status in a gene-dosage

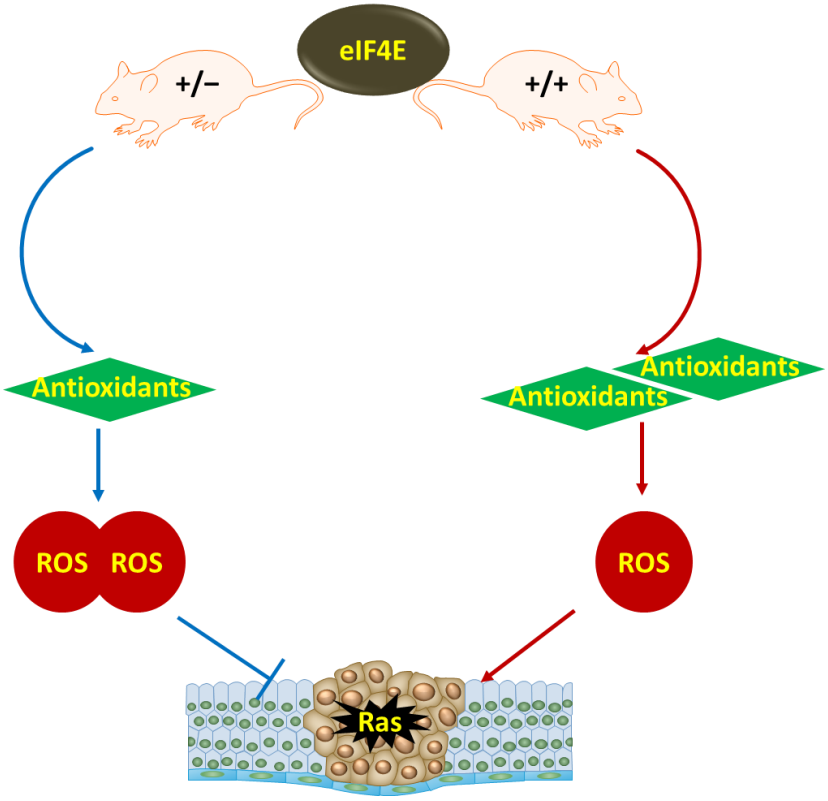

FIGURE 3. The 'eIF4E-antioxidnts/ROS' axis in oncogenic transformation and tumorigenesis. See text (Section 3) for detailed discussion. This scheme is based on Ref. [31].

dependent manner [31]. This is the first comprehensive study that reveals a linkage between a crucial translational regulator and tumorigenesis via a cellular redox-based bridge.

By generating an eIF4E haploinsufficient mouse, Truitt et al. showed that a 50% reduction in eIF4E expression, while compatible with normal development and global protein synthesis, significantly impeded oncogenic cellular transformation [31]. They then performed genome-wide translational profiling and uncovered a translational program induced by oncogenic transformation and revealed that the dose of eIF4E was essential for translating mRNAs that regulate ROS homeostasis, including especially the glutathione system [31]. The glutathione system consists of glutathione and enzymes that generate glutathione or using glutathione as a substrate for ROS detoxification, and is a major antioxidant defense involved in regulating cellular ROS and redox homeostasis [32]. The finding, that eIF4E upregulated the expression of the antioxidant defense in a gene-dosage dependent manner during oncogenic transformation and tumorigenesis, highlights an important role for cellular antioxidant status in promoting tumorigenesis [31, 33]. Cancer cells typically produce higher levels of ROS than normal cells. Such elevated ROS levels, when they are moderate, are used as signaling molecules to promote cancer cell growth. On the other hand, when the levels of ROS go beyond moderate, these reactive species may actually become cytotoxic and thus inhibit cancer cell growth [34]. Indeed, either over expression of endogenous antioxidants or supplementation of exogenous antioxidants has been found to promote tumorigenesis and cancer metastasis in certain experimental models [33, 35]. In line with the above notion, Truitt et al. demonstrated that eIF4E haploinsufficiency rendered $KRas^{LA2}$ tumors remarkably susceptible to a pharma-

cological agent-induced ROS formation compared to wide-type tumors, resulting in significant reductions in tumor volumes [31]. This finding is of important implications with regard to the use of ROS-producing drugs to treat cancer with augmented eIF4E activity. In this regard, ROS-producing agents have been emerging as novel effective modalities for cancer treatment [34].

The elegant study by Truitt et al. provided another excellent scenario whereby too much antioxidant defense might promote cancer development (**Figure 3**), which is in contrast to the conventional belief that antioxidants are always beneficial to health. This notion, on the one hand, reveals the complexity of ROS biology, and on the other hand, points to the need for taking such a complexity into consideration when devising strategies for the intervention of diseases involving an ROS-dependent mechanism.

4. CONCLUDING REMARKS

The cutting edge findings reported in the four Cell articles discussed in this 'ROS Research Highlights' paper adds to the continued excitement about the cell biology of ROS, reactive species that are derived from our utilization of molecular oxygen. These innovative studies not only increase our current understanding of ROS bioscience, but also provide unique opportunities for future studies to translate such cutting edge scientific knowledge into modalities that can be used to benefit human health. It is hoped that introducing and discussing these high-profile research discoveries published in Cell as well as other high influential journals would help stimulate high-impact innovative research toward deciphering the biological enigmas of these ubiquitous oxygen-containing reactive species as well as the entities (e.g., antioxidant molecules) involved in regulating their cellular homeostasis.

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