Introduction and reconciliation of the ROS and aging paradoxes.

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Abstract

Researchers in the aging field are getting headache by the ROS and aging paradoxes. There are both experiments supporting and challenging the role of ROS in aging, especially in studies using C. elegans as the model. The view that ROS are beneficial and can slow down aging is getting popular. In this paper we proposed explanations on how these contradictory conclusions were generated by taking into account previously unnoticed factors including the excessive response, retardation of growth, and the reliability of ROS measuring approaches. We believe that aging is encoded by genes or DNA and influenced synthetically by pro-aging factors like ROS and unknown side effects, and anti-aging factors such as particular protective responses. Any pro- or anti-aging roles should be ascribed to the “net” effects rather than that of one particular factor like ROS. From this point of view the ROS and aging paradoxes can be reasonably explained and there are no contradictions with the oxidative stress theory of aging. Nevertheless, we also believe that ROS have limited role in aging considering the prime outcome of evolution or natural selection should be increased adaption to the environment rather than long lifespan. The increase of longevity observed in model organisms may be by product of retrograde responses motivated against certain circumstances.

Keywords: ROS, Aging, Synthetic effect, Pro-aging factors, Anti-aging factors.

Introduction

Reactive oxygen species (ROS) are highly reactive chemical species containing oxygen and are mainly generated in mitochondria during respiration [1]. ROS react with macromolecules including proteins, lipids, and nucleotides [1,2]. The oxidative stress theory of aging speculated that damages caused by ROS would lead to cellular dysfunctions and aging [3]. There are experiments both supporting and challenging the theory, and recently the view that ROS are beneficial and can slow down aging is getting more and more popular [4-7]. In this paper the ROS and aging paradoxes are summarized and reconciled, which we hope will make things clear in correlated areas.

Description of the ROS and aging paradoxes

The “ROS and aging paradoxes” are mainly reflected in the following pairs of contradictory reports:

a. The antioxidant resveratrol (RSV) slows down aging in both invertebrates and vertebrates, and the beneficial effects of other antioxidants including N-acetylcysteine (NAC), vitamin C, reduced glutathione, thioproline, and platinum nanoparticle are also reported [8-12]. However, the pro-oxidant paraquat (PQ) is also considered to have anti-aging effects [14-16].

b. Over-expression of the major antioxidant enzyme SOD-1 increases and deletion of the thioredoxin TRX-1 decreases longevity in C. elegans [14-16]. Mice with deletion of sod-1 have accelerated aging phenotype correlated with increased cellular senescence, and are ideal models for human frailty [17]. But there are also reports showed that deletion all five sod genes in C. elegans did not decrease lifespan and over-expression of sod-2 even increased it [18,19].

c. Genetic or environmental perturbations that prolong lifespan including reduced insulin/IGF-1 signaling (IIS), mitochondrial dysfunctions, and dietary restriction (DR) usually activate multiple protective responses such as increased expression of antioxidant and xenobiotic detoxification enzymes, increased autophagy, and other unknown adjustments [20-22].

The DAF-16/FoxO3a-dependent longevity signal was also shown to be initiated by antioxidants [13]. Consistenting with the up-regulation of antioxidant enzymes ROS are found decreased in worms chronically treated with sub-lethal levels of the pro-oxidant paraquat, and in those with deficiencies of genes encoding the mitochondrial subunits NUO-6 or CCO-1 [13,24-26]. However, some studies reported increased ROS under the above mentioned conditions [27].

Researchers are getting puzzled by these paradoxes and are beginning to describe ROS as a beneficial player in aging. We believe that if the pro-longevity roles be attributed to the synthetic effects of secondary responses including the activation of protective mechanism, retardation of growth, and other unknown factors, rather than that of ROS, most if not all of these paradoxes would be reasonably reconciled. Some
paradoxes may also be originated from the unreliability of ROS
detecting approaches explained in the following words.

**Reconciliation of the ROS and aging paradoxes**

The pro-longevity roles should be ascribed to the motivated
protective mechanisms rather than ROS In response to increased
ROS levels, protective mechanisms are excessively activated
including up-regulated expression of antioxidant enzymes,
increased autophagy, and other unknown adjustments. The
persistent and excessive activation of these mechanisms may
lead to decrease of ROS in the long term as described by the
excessive response model [25,28,29]. Among these mechanisms,
the antioxidant enzymes and autophagy related pathways are
reported to have anti-aging effects [14-16]. If the protective
mechanisms are anti-aging then ROS are likely to be the opposite
because the former are motivated as countermeasures against the
latter. Just like we consider the immune response but not virus
as being beneficial, the pro-longevity effects, if any, should also
be ascribed to the protective mechanisms rather than ROS. If so,
most of the paradoxes would be reasonable reconciled and the
seemingly contradictive studies are actually in accordance with
the oxidative theory of aging. Growth retardation should be
taken into account sometimes Deficiencies of either cytosolic or
mitochondrial SODs usually lead to reduced lifespan, sickness,
and lethality in yeasts, flies, and mice [30-32]. But deletion of
all sods genes in C. elegans does not decrease longevity and
knockout of the mitochondrial localized superoxide dismutase
sod-2 increases it [24,25]. Although the sod quintuple mutant
worms are considered to have normal lifespan they exhibit
slow development, reduced fertility, slower defecation cycle,
decreased movement, and increased sensitivity to oxidative
stresses.

The sod quintuple mutant and sod-2 single mutant worms both
need more than two days to finish growth compared to wild
type [18,19]. If growth retardation was taken into account the
quintuple mutant worms’ lifespan would decrease and the pro-
longevity effect of deletion of sod-2 would diminish. In addition,
sod-2 deletion was reported to have no effect on lifespan in other
studies [4]. Unlike vertebrates, it is quite common for C. elegans
to retard growth under detrimental conditions and the retardation
is usually accompanied with sickness or fragility. Similarly, the
sod mutants are also sick and vulnerable [18,19]. Therefore,
some of the paradoxes are produced due to the overlook of
growth retardation or sickness. It should be cautious to use ROS
data obtained from tissue lysates or isolated organelles.

The reliability of ROS detecting approaches is prerequisite
for getting any reliable conclusion. Some of the ROS and
aging paradoxes indeed arise from opposite ROS results. For
example, ROS are found to be decreased in worms with
mutations of the mitochondrial respiratory chain subunits nuo-6
or isp-1, and in worms treated chronically with un-lethal level
of paraquat [13,24,25]. But others reported increased ROS by
similar treatments [27]. The contradictions should be caused by
big variations of ROS data obtained from isolated mitochondria.
In vitro ROS measurements are unreliable due to the following
reasons: Firstly, Unlike DNA, RNA, proteins, lipids, and other
stable molecules, ROS are unstable, highly reactive, and change
rapidly. ROS are mainly generated as by-products of mitochondrial
respiration, and any perturbations of the concentrations of
substrates such as pyruvate, oxygen, ADP, and others would
dramatically affect the generation or degradation of ROS.
Isolated mitochondria are in un-physiological state and the
concentrations of substrates for metabolism are all altered
[32]. As the by-products, ROS levels must also change
immediately. Secondly, the lysis procedures such as sonication
and homogenization would generate heat themselves, disrupt
mitochondrial structures, and lead to intracellular or intra-
organelle release of iron ions, all of which would generate extra
ROS. In addition, Fe2+ ions mediated Fenton reaction is an
important source of ROS. It is thus recommended to measure
ROS in living worms instead of in worm lysates or isolated
organelles [24]. Finally, the so called “Thinking Set” may also
influence the reliability of ROS detection.

To some researchers, it seems to be logical that pro-oxidant
treatments or disruptions of mitochondria functions would
increase ROS. But in fact it is not the case, although transient
pro-oxidant stresses increase ROS, the chronic treatments lead to
opposite results due to the excessive response of the antioxidant
systems [24,25]. In worms with deficiencies of nuo-6 or cco-1
ROS are also reduced [24]. Consistently, elevated ROS levels
due to increased respiration would activate antioxidant enzymes
and further decrease ROS in the long term [28]. If the ROS data
obtained are opposite, it is not surprise that some of the ROS
and aging paradoxes are produced. ROS should be more closely
 correlated with metabolism and may have limited role in aging

The prime outcome of evolution or natural selection should be
enhanced adaption to environment rather than long lifespan.
ROS levels should also be tuned to adjust to metabolism.
Mitochondria are the main site for ATP production and ROS
generation, suggesting the close correlation between metabolism
and ROS. Consistently, ROS participate in cell respiration as
intermediate products and act as signals in glucose stimulated
insulin secretion [33,34]. They are widely distributed in cells
and unlikely play distinctive, direct, and prime roles in aging.
Aging should be encoded by genes or DNA and ROS may have
limited impact on it, which may be one of the reasons for the
origin of the paradoxes mentioned above

**Conclusion**

In conclusion, exogenous or endogenous pro-oxidant stresses
would activate adaptive responses including the increased
expression of antioxidant enzymes, increased autophagy,
and other yet to be identified mechanisms, among which the
antioxidant enzymes and autophagy are reported to have anti-
aging effects. The excessive response model states that when
pro-oxidant capacity goes high the antioxidant capacity
will decrease and ROS levels will be observed [25-38]. It is thus not surprise that increased longevity is observed
under proper level of pro-oxidant stresses. The protective
mechanisms are activated by and tackle against the rise of
ROS. If the formers are anti-aging then ROS should be pro-
aging, and aging should be affected synthetically by pro-aging
factors such as ROS and unknown side effects, and anti-aging
factors such as antioxidants, autophagy, and others (Figure 1).
Therefore, most if not all of the ROS and aging paradoxes could be reasonably reconciled and we also believe that ROS have limited roles in aging considering that long lifespan is not the prime goal of evolution or natural selection. The increase of longevity observed in model organisms should be by-product of retrograde responses, of which the main product may be increased adaption to adverse conditions. We are confident that the perspective proposed here will increase the understanding of the relationship between ROS and aging.

**Conflict of interests**

The authors declare that there is no conflict of interest.

**Funding**

This work was supported by the National Natural Science Foundation of China [Grant numbers 81200253, 81570760, and 31771283]; the National Key Research and Development Program of China [Grant numbers 2017YFA0103900, 2017YFA0103902, and 2016YFA0102200]; One Thousand Youth Talents Program of China to C. Zhang; the Program for Professor of Special Appointment (Eastern Scholar) at Shanghai Institutions of Higher Learning [Grant number A11323]; the Shanghai Rising-Star Program [Grant number 15QA1403600]; and the Fundamental Research Funds for the Central Universities of Tongji University.

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**Figure 1:** Aging is affected synthetically by pro- and anti-aging factors. (A) Under adverse conditions such as increased pro-oxidant stresses or cellular dysfunctions ROS and other side effects are produced. Retrograde signaling pathways are activated and downstream secondary responses including up-regulation of antioxidant enzymes, increased autophagy, and others are persistently and excessively motivated to fight against ROS or other side effects. (B) If the synthetic effect of pro-aging factors overshadows that of anti-aging factors aging process will be accelerated. Otherwise, the aging process will be decelerated.
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