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Mitochondria targeting by environmental stressors: implications for redox cellular signaling

Chuck Blajszczak and Marcelo G. Bonini

Departments of Medicine and Pathology

Abstract

Mitochondria are cellular powerhouses as well as metabolic and signaling hubs, regulating diverse cellular functions from basic physiology to phenotypic fate determination. It is widely accepted that reactive oxygen species (ROS) generated in mitochondria participate in the regulation of cellular signaling and that there are mitochondria which operate at a high ROS baseline. However, how mitochondria adapt to persistently high ROS states as well as to environmental stressors that disturb the redox balance is not completely understood. Here we will review some of the current concepts regarding how mitochondria resist oxidative damage, how they are replaced when oxidative damage is excessive to an extent that compromises function, and what is the effect of some environmental toxicants (i.e. heavy metals) on the regulation of mitochondrial ROS (mtROS) production which are linked to their toxic effects on cells and tissues.

INTRODUCTION

Initially regarded as damaging by-products of respiration, reactive oxygen species (ROS) emerged in the 1990s as critical agents of cellular signaling and regulators of cellular functions^[1–3]. It is now clear that among the many physiological sources of ROS, mitochondria play a critical role. Not only can mitochondria undisputedly produce large amounts of ROS but they also couple ROS generation with the nearly immediate engagement of metabolic responses from the cell^[4–6]. By being both a center of ROS production and the major cellular metabolic hub, mitochondria serve as a major junction regulating intra- and inter-cellular communications and adaptations of cells to their immediate microenvironment as well as systemic changes.

Studies over the last two decades have indicated that variations in the generation rate of mitochondrial ROS (mtROS) and its subsequent diffusion out of mitochondria regulate critical cellular processes such as proliferation^[7, 8], quiescence^[9, 10], physiologic function^[10–13]. Additionally, ROS influences if the cells will assume a fully functional differentiated state, remain or return to an “stemness” program^[14–17]. Hence, any exogenous agent interfering with the ability of mitochondria to regulate ROS production and/or neutralization is likely to ultimately affect cellular phenotype and function. These insults deregulate both the mitochondrial energetic metabolism and ROS signaling homeostasis.

[§]Correspondence to: Marcelo G. Bonini, Ph.D., Departments of Medicine, Pathology and Pharmacology, University of Illinois at Chicago, UIC, 909 S. Wolcott Ave, COMRB 1131, Chicago, IL, 60612, Phone – (312) 355-5948 mbonini@uic.edu.

In this mini-review we will focus on a few major questions still puzzling the field despite intensive investigation: 1) Where and how ROS are generated in mitochondria; 2) What are some of the most relevant mechanisms of ROS neutralization in mitochondria; 3) How toxicants may interfere with these processes and how mitochondria can be protected or replaced; 4) Finally, what are some of the potential consequences of dysregulated mtROS for human disease.

Sources of mtROS

Most of the studies on the mechanisms of ROS formation in mitochondria agree that the electron transport chain (ETC) is the major source and that superoxide radical anion is the primary ROS produced in mitochondria^[18–20]. Though other sources have been identified (i.e. p66shc^[21, 22], Nox4^[23–25], etc.), ETC-generated ROS seem to be central for the promotion of the signaling effects of mtROS^[26]. Within the ETC constituents, complexes I and III appear to be the major sites of ROS generation^[20, 27–29]. This production of ROS is amplified by the blockade of electron flow as well as the inhibition of electrochemical potential dissipation which is attained either by the inhibition of ETC complexes^[30, 31] or F₀F₁-ATPase. The increase in ROS attained by the inhibition of some of the ETC complexes or ATPase function can normally be released by uncoupling agents such as FCCP or 2,4-DNP^[32]. Thus, when electrons accumulate within the ETC complexes, more ROS are generated. Additionally, a role for complex II as a regulator of ROS production from complex I has been proposed^[20]. Complex II creates a backflow of electrons from succinate dehydrogenase (complex II), making this the only enzymatic system involved in both the Krebs cycle and the ETC simultaneously. It appears that under conditions that favor succinate accumulation and oxidation (i.e. ischemia/reperfusion), a torrent of electrons entering the ETC through complex II overwhelms the electron transferring capacity of carriers and complexes downstream of it, leading to the backflow electrons towards complex I. Therefore, toxicants interfering with the metabolism of succinate and the transfer of electrons through the complexes have an enormous potential to disrupt the delicate balance between ROS production and neutralization in mitochondria.

Another emerging theory is that the source of ROS fundamentally impacts the outcome. Studies indicate that ROS from complex III are critical for cellular fate decisions by progenitor cells, such as maintaining stem-like traits or during adipocytic differentiation^[33]. Conversely, ROS from complex I contribute to both oxidative damage following ischemia-reperfusion challenge and to the extension of the life span in simpler eukaryotic organisms^[34]. New evidence indicates that the route to ROS generation is important and that the redirection of electron flow in mitochondria may represent a regulatory pathway towards specific outcomes. This notion which seems to be gaining ground is counter-intuitive, given the reactive nature and intrinsic lack of selectivity of ROS. Hence, progress towards elucidating the molecular mechanisms guiding the reactivity of ROS in such complex contexts is needed so effects and cellular outcomes of ROS signaling will become more predictable and potentially controllable.

Neutralizing ROS – how can mitochondria stand the heat?

It is becoming increasingly clear that under certain conditions (i.e. certain cancers) mitochondria continuously function at a higher level of ROS production. Mitochondrial biomolecules are primary targets of ROS formed within, because ROS are species that react at or near their site of formation [35, 36]. How, then, is it possible that mitochondria can function under such unfavorable conditions?

One possibility is that mitochondria functioning at a higher rate of ROS production are better equipped to eliminate ROS by harboring elevated levels of enzymes capable of handling superoxide radicals and H₂O₂. This possibility seems to be supported by the finding of high levels of MnSOD which function at an elevated ROS state in certain tumors [12, 37]. However, while high MnSOD would be expected to reduce superoxide radical accumulation, H₂O₂ levels would simultaneously rise unless other enzymes, such as peroxiredoxins or glutathione peroxidases, are also elevated. These other enzymes must also be equipped to reduce H₂O₂ to water using thioredoxin or glutathione, respectively, as substrates. Hence, the fate of mtROS will depend on a fine balance of enzymes and available cofactors regulating the rate of decay of superoxide to H₂O₂ and the detoxification of H₂O₂ itself.

Another possibility is that mitochondria functioning at high ROS production rates will be recycled more vigorously through the combination of mitophagy^[38, 39] and mitochondrial biogenesis^[40, 41]. Mitophagy is the process by which worn out mitochondria are destroyed. Similarly to autophagy, mitophagy is perceived as a mechanism that allows the recycling and repurposing of basic mitochondrial components such as lipids and amino acids. Mitochondrial biogenesis is the process by which new mitochondria are generated. The connection between higher ROS formation in cancer and mitochondria recycling has been explored in detail elsewhere (for a review please see [40, 42, 43]).

Mitochondria DNA integrity is important to suppress ROS

When considering factors such as mitochondria's ability to handle ROS or how efficiently they are recycled, a critical parameter determining the production of ROS in mitochondria is the integrity of mtDNA^[44–46]. Most of the mitochondrial proteome is codified by the nuclear genome. However, there is a growing list of proteins and bioactive peptides which directly affect the ETC both in terms of energetic efficiency and electron leakage to make ROS. Before 2001, it was believed that 13 proteins were codified by the mtDNA, all being components of different complexes of the ETC. However, in 2001 a 14^[th] peptide humanin, was discovered^[47, 48]. It is becoming clear that humanin, as well as other mitochondrial peptides^[49], belong to a family of mitochondria-encoded hormones which regulate nutrient disposition and cellular bioenergetics at distant sites. Damage to mtDNA results in altered expression and/or the expression of dysfunctional components of the ETC which are less capable of regulating the transfer of electrons down the chain. Accumulation of mtDNA damage is normally translated into decreased ATP production and increased ROS per NADH-equivalent. Since mtDNA is a template for its own replication, the accumulation of oxidative damage might reduce the overall quality of templates. This could lead to an

increase in defective or mutated copies that get passed onto newly formed mitochondria. It is possible that a threshold of damage exists beyond which mitochondrial biogenesis becomes ineffective as a means to repopulate the “healthy” mitochondria pool.

mtROS and the cellular metabolism

It is now well-established that mitochondria regulate the cellular metabolism both directly, via the processing of numerous metabolites, or indirectly, via regulating the transcription of genes that regulate the metabolism. The latter involves the elevation of ROS production and the activation of transcriptional networks which normally respond to changes in oxygen levels, including hypoxia-induced transcription factors (HIF)^[50]. HIFs are broad-acting transcription factors responsible for the expression of a wide-variety of genes influencing the cellular metabolism as well as phenotypic transitions and malignant transformation^[51, 52]. Although there are 3 HIF factors, most attention has been given to HIF1 and HIF2^[53]. HIFs are formed by the dimerization of a variable subunit (alpha-subunits) and the constitutively expressed beta-subunit (HIF1 β /ARNT). Although HIF1 and HIF2 have redundant targets, it is the binding of the alpha-subunit that ultimately determines the target gene preference of the transcriptional program. HIF1 α is normally associated with the activation of glycolysis and angiogenesis, and HIF2 α is mostly associated with phenotypic reprogramming and stemness^[54, 55]. Both HIF1 α and HIF2 α are continuously made, and while oxygen is available, are continuously degraded by prolyl-hydroxylase (PHD) enzymes. PHDs are inhibited by ROS, leading to well-established role of ROS as activators of HIFs^[50]. However, since both HIFs are regulated similarly how is it mechanistically possible to activate either program specifically? Although a definitive answer will demand additional insight from upcoming studies, a possibility emerged from the works of Powis and colleagues on a factor called hypoxia-associated factor (HAF)^[56]. The investigators found that HAF can bind both HIF1 α and HIF2 α , but while binding to HIF1 α destabilizes the protein, binding to HIF2 α promotes its stability and the activity of HIF2 transcription factor. It is possible that the accumulation of HAF serves as a switch in HIF programs from a more transient HIF1 α -driven signal, for short-term adaptation to hypoxia, to a more lasting HIF2 α -driven program, associated with phenotypic reprogramming.

Consistent with these findings, our own published studies have indicated that changes in the metabolism of mtROS caused by an upregulation of MnSOD expression promotes lasting metabolic changes^[12]. For instance, we have found that an increase in H₂O₂ in cells stably overexpressing MnSOD promotes the activation of glycolysis via the activation of CaMKII and AMPK. We propose that the activation of CaMKII by oxidation is a mechanism that bypasses the regulation of glycolysis by the ETC. This could occur because the glycolytic flow is normally controlled by ATP, which in turn is produced in large amounts by the ETC. High ATP levels inhibit enzymes in the glycolytic pathway which is the primary source of pyruvate, a metabolite used by mitochondria to power the ETC. This circuit establishes an auto-regulatory mechanism between the ETC and glycolysis which is disrupted when AMPK is activated by H₂O₂ (via CaMKII). We propose that increased mtROS and H₂O₂ promote the metabolic uncoupling of glycolysis and the ETC by at least two mechanisms involving HIFs and the activation of glycolysis directly by the CaMKII-activated AMPK.

Heavy metals, metalloids, and mtROS – what are the connections?

It is well-known that heavy metals and metalloids such as inorganic arsenic (iAs/As(III)) promote changes in mtROS production and metabolism, thereby impacting cell signaling and shaping of cellular phenotypes^[57–59]. Most elements in this class exert their toxic effects by directly binding and modifying the catalytic activity of enzymes involved in electron transfer, redox reactions, protein synthesis and DNA-repair^[60–63]. For this review, we will focus on the ability of certain metals to modify the redox state of the cells by 1) diminishing the ability of the cell to buffer ROS formation; 2) increasing or promoting ROS formation; and 3) amplifying ROS formation.

Heavy Metals and ROS – how do metals inhibit ROS detoxifying enzymes?

Most heavy metals and some metalloids bind sulfur and selenium with very high affinity. Metals of relevance for human health such as cadmium (Cd), mercury (Hg), lead (Pb) and Arsenic (As) have also been shown to bind with high affinity to thiol/selenol residues in peptides^[64–68] and proteins, with their binding being normally sufficient to inhibit the enzymes' activities. Most enzymes catalyzing ROS detoxification are dependent on either an active site thiol or selenol – glutathione peroxidase and peroxiredoxin isoenzymes are classic examples. The binding of Cd²⁺, Hg²⁺, Pb²⁺ or As³⁺ as well as chromium and vanadium compounds to the sulfur or selenium atoms in these proteins blocks the ability of these redox active moieties from interacting with H₂O₂ and other peroxides, resulting in the accumulation of these ROS. Also, since the affinity constant between Sulfur/Selenium and heavy metals is relatively high, the dissociation of these metals from the enzymes they interact with is fairly uncommon. This affinity results in the persistent inhibition of these enzymes and the need to replace the enzyme pool at the expense of cellular energy and biomass. In addition to directly affecting glutathione peroxidases (GPx) and peroxiredoxins (Prx), heavy metals can also impact these enzymes' activities indirectly. GPx activity is supported by glutathione, and Prx depend on thioredoxin which are themselves reactive towards heavy metals and Arsenic. In addition, enzymes that recycle these compounds such as glutathione and thioredoxin reductases are also targets. Therefore, from the standpoint of limiting the ability to detoxify ROS, heavy metals and metalloids work through several different mechanisms which include inhibiting the effector enzymes, limiting cofactor availability or the capacity them.

Heavy Metals and ROS – how do metals increase ROS formation?

Mechanisms of increased ROS formation by redox active metals also vary and can be direct or indirect. Some metals with different valence states such as iron, copper, and chromium, can redox cycle (i.e. be reduced by cellular antioxidants such as NADPH, BH₄GSH) and then react with oxygen directly producing ROS (please see reactions 1–3)^[74].

Alternatively, several heavy metals and metalloids act as inhibitors of enzymes that regulate the activity of effectors of ROS production and detoxification (some examples were discussed above). Another component involved in regulating ROS is the Sirtuin 3/MnSOD axis. Sirtuin 3 (Sirt3) is the primary mitochondrial deacetylase acting upon MnSOD^[75]. Acetylation of two critical lysine residues (K68, K122) on MnSOD inhibits the enzyme in regards to its ability to scavenge superoxide radicals^[75, 76]. Hence, Sirt3 acts as an activator of MnSOD superoxide dismutase activity. A recent study indicated that arsenic reduces Sirt3 expression^[77], suggesting arsenic can increase superoxide radical levels by inhibiting MnSOD deacetylation. Sirt3 is also responsible for the deacetylation and activation of several complexes of the ETC^[78]. Thus, the inhibition of Sirt3 is likely to alter the ROS/ATP ratio produced per unit of NADH in favor of ROS. This example about Sirt3/MnSOD illustrates how intricate and far-reaching the effects of heavy metal exposure can be. It also demonstrates how many different systems may be affected by inhibiting a single pathway involved both in the metabolic regulation of electron flow through the ETC and the mitigation of superoxide radical production in mitochondria.

Inflammation: A mechanism by which Heavy Metals may amplify oxidative stress

Inflammation activates numerous mechanisms of ROS production in many cell types and is itself regulated by ROS^[79, 80]. It has been reported that inflammation is regulated by thioredoxin^[81] which can be depleted by heavy metals (as reviewed above). Consequently, the depletion of thioredoxin is a powerful pro-inflammatory stimulus that can produce oxidative stress and oxidative damage. In addition, we have recently found that the pro-inflammatory activity of NFκB can be enhanced in the nucleus by thiol-modifying agents via the inhibition of suppressor of cytokine signaling-1 (SOCS1)^[82]. SOCS1 is a negative regulator of NFκB which targets the catalytic p65 subunit to degradation^[83]. Without p65, NFκB cannot promote the transcription of pro-inflammatory genes. Therefore, SOCS1 suppresses or terminates inflammation indirectly restricting oxidant production and oxidative stress. Our previous research has demonstrated that SOCS1 harbors two critical cysteine residues (Cys 147 and Cys 179)^[82] which are susceptible to oxidative modification and are likely reactive against heavy metals with a high affinity for thiols. The blockade of these thiol residues renders SOCS1 incapable of binding p65 and targeting it for degradation, so it is possible that SOCS1 inhibition is an additional mechanism by which some metals induce inflammation. Although further research is needed to establish this concept, most available data surrounding heavy metal biochemistry and SOCS1 regulation

of inflammation suggest that inactivation of SOCS1 is a plausible mechanism by which some metals could promote the pro-inflammatory activity of NF κ B.

Conclusion

Reactive oxygen species (ROS) are critical regulators of the cellular metabolism and phenotypic transitions, and therefore play major roles in health and disease. Heavy metals and metalloids are powerful regulators of ROS formation which act via several different mechanisms (Figure 1). However, the high reactivity of heavy metals with thiol groups as well as the irreversibility of these reactions suggests that thiolate/selenol-dependent protein inhibition is a major aspect of metal toxicity, including the acknowledged effects of some heavy metals in promoting or amplifying oxidative stress. Research in this area may promote a better understanding of how the interactions of certain populations within their environment change critical molecular switches in cells, including those regulated by mitochondria and ROS. It is also likely to lead to better and improved policies, mitigation strategies, and clinical approaches which will be helpful to people affected by illnesses initiated by ROS from endogenous or exogenous sources.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Rhee SG. Redox signaling: hydrogen peroxide as intracellular messenger. *Exp Mol Med*. 1999; 31(2):53–9. PubMed PMID: 10410302. DOI: 10.1038/emmm.1999.9 [PubMed: 10410302]
2. Hensley K, Robinson KA, Gabbita SP, Salsman S, Floyd RA. Reactive oxygen species, cell signaling, and cell injury. *Free Radic Biol Med*. 2000; 28(10):1456–62. PubMed PMID: 10927169. [PubMed: 10927169]
3. Finkel T. Redox-dependent signal transduction. *FEBS Lett*. 2000; 476(1–2):52–4. PubMed PMID: 10878249. [PubMed: 10878249]
4. Chandel NS. Mitochondria as signaling organelles. *BMC Biol*. 2014; 12:34. PubMed PMID: 24884669; PubMed Central PMCID: PMC4035690. doi: 10.1186/1741-7007-12-34 [PubMed: 24884669]
5. Brookes PS. Mitochondrial H(+) leak and ROS generation: an odd couple. *Free Radic Biol Med*. 2005; 38(1):12–23. PubMed PMID: 15589367. DOI: 10.1016/j.freeradbiomed.2004.10.016 [PubMed: 15589367]
6. Zhang DX, Gutterman DD. Mitochondrial reactive oxygen species-mediated signaling in endothelial cells. *Am J Physiol Heart Circ Physiol*. 2007; 292(5):H2023–31. PubMed PMID: 17237240. DOI: 10.1152/ajpheart.01283.2006 [PubMed: 17237240]
7. Wang M, Kirk JS, Venkataraman S, Domann FE, Zhang HJ, Schafer FQ, et al. Manganese superoxide dismutase suppresses hypoxic induction of hypoxia-inducible factor-1 α and vascular endothelial growth factor. *Oncogene*. 2005; 24(55):8154–66. PubMed PMID: 16170370. DOI: 10.1038/sj.onc.1208986 [PubMed: 16170370]

8. Diebold L, Chandel NS. Mitochondrial ROS regulation of proliferating cells. *Free Radic Biol Med*. 2016; 100:86–93. PubMed PMID: 27154978. DOI: 10.1016/j.freeradbiomed.2016.04.198 [PubMed: 27154978]
9. Sarsour EH, Venkataraman S, Kalen AL, Oberley LW, Goswami PC. Manganese superoxide dismutase activity regulates transitions between quiescent and proliferative growth. *Aging Cell*. 2008; 7(3):405–17. PubMed PMID: 18331617; PubMed Central PMCID: PMCPMC2538945. DOI: 10.1111/j.1474-9726.2008.00384.x [PubMed: 18331617]
10. Menon SG, Sarsour EH, Kalen AL, Venkataraman S, Hitchler MJ, Domann FE, et al. Superoxide signaling mediates N-acetyl-L-cysteine-induced G1 arrest: regulatory role of cyclin D1 and manganese superoxide dismutase. *Cancer Res*. 2007; 67(13):6392–9. PubMed PMID: 17616699. DOI: 10.1158/0008-5472.CAN-07-0225 [PubMed: 17616699]
11. Hart PC, Ratti BA, Mao M, Ansenberger-Fricano K, Shajahan-Haq AN, Tyner AL, et al. Caveolin-1 regulates cancer cell metabolism via scavenging Nrf2 and suppressing MnSOD-driven glycolysis. *Oncotarget*. 2016; 7(1):308–22. PubMed PMID: 26543228; PubMed Central PMCID: PMCPMC4808000. DOI: 10.18632/oncotarget.5687 [PubMed: 26543228]
12. Hart PC, Mao M, de Abreu AL, Ansenberger-Fricano K, Ekoue DN, Ganini D, et al. MnSOD upregulation sustains the Warburg effect via mitochondrial ROS and AMPK-dependent signalling in cancer. *Nat Commun*. 2015; 6:6053. PubMed PMID: 25651975; PubMed Central PMCID: PMCPMC4319569. doi: 10.1038/ncomms7053 [PubMed: 25651975]
13. Ekoue DN, He C, Diamond AM, Bonini MG. Manganese superoxide dismutase and glutathione peroxidase-1 contribute to the rise and fall of mitochondrial reactive oxygen species which drive oncogenesis. *Biochim Biophys Acta*. 2017; PubMed PMID: 28087256. doi: 10.1016/j.bbabbio.2017.01.006
14. Loo SY, Hirpara JL, Pandey V, Tan TZ, Yap CT, Lobie PE, et al. Manganese Superoxide Dismutase Expression Regulates the Switch Between an Epithelial and a Mesenchymal-Like Phenotype in Breast Carcinoma. *Antioxid Redox Signal*. 2016; 25(6):283–99. PubMed PMID: 27400860; PubMed Central PMCID: PMCPMC4991580. DOI: 10.1089/ars.2015.6524 [PubMed: 27400860]
15. Prasanphanich AF, Arencibia CA, Kemp ML. Redox processes inform multivariate transdifferentiation trajectories associated with TGFbeta-induced epithelial-mesenchymal transition. *Free Radic Biol Med*. 2014; 76:1–13. PubMed PMID: 25088330; PubMed Central PMCID: PMCPMC4254148. DOI: 10.1016/j.freeradbiomed.2014.07.032 [PubMed: 25088330]
16. Zhou G, Meng S, Li Y, Ghebre YT, Cooke JP. Optimal ROS Signaling Is Critical for Nuclear Reprogramming. *Cell Rep*. 2016; 15(5):919–25. PubMed PMID: 27117405; PubMed Central PMCID: PMCPMC4856580. DOI: 10.1016/j.celrep.2016.03.084 [PubMed: 27117405]
17. Lee S, Tak E, Lee J, Rashid MA, Murphy MP, Ha J, et al. Mitochondrial H2O2 generated from electron transport chain complex I stimulates muscle differentiation. *Cell Res*. 2011; 21(5):817–34. PubMed PMID: 21445095; PubMed Central PMCID: PMCPMC3203677. DOI: 10.1038/cr.2011.55 [PubMed: 21445095]
18. Lopez-Fabuel I, Le Douce J, Logan A, James AM, Bonvento G, Murphy MP, et al. Complex I assembly into supercomplexes determines differential mitochondrial ROS production in neurons and astrocytes. *Proc Natl Acad Sci U S A*. 2016; 113(46):13063–8. PubMed PMID: 27799543; PubMed Central PMCID: PMCPMC5135366. DOI: 10.1073/pnas.1613701113 [PubMed: 27799543]
19. Koopman WJ, Verkaart S, Visch HJ, van der Westhuizen FH, Murphy MP, van den Heuvel LW, et al. Inhibition of complex I of the electron transport chain causes O2-.-mediated mitochondrial outgrowth. *Am J Physiol Cell Physiol*. 2005; 288(6):C1440–50. PubMed PMID: 15647387. DOI: 10.1152/ajpcell.00607.2004 [PubMed: 15647387]
20. Murphy MP. How mitochondria produce reactive oxygen species. *Biochem J*. 2009; 417(1):1–13. PubMed PMID: 19061483; PubMed Central PMCID: PMCPMC2605959. DOI: 10.1042/BJ20081386 [PubMed: 19061483]
21. Giorgio M, Migliaccio E, Orsini F, Paolucci D, Moroni M, Contursi C, et al. Electron transfer between cytochrome c and p66Shc generates reactive oxygen species that trigger mitochondrial apoptosis. *Cell*. 2005; 122(2):221–33. PubMed PMID: 16051147. DOI: 10.1016/j.cell.2005.05.011 [PubMed: 16051147]

22. Veeramani S, Yuan TC, Lin FF, Lin MF. Mitochondrial redox signaling by p66Shc is involved in regulating androgenic growth stimulation of human prostate cancer cells. *Oncogene*. 2008; 27(37): 5057–68. PubMed PMID: 18504439; PubMed Central PMCID: PMC2776635. DOI: 10.1038/onc.2008.143 [PubMed: 18504439]
23. Zheleznova NN, Yang C, Cowley AW Jr. Role of Nox4 and p67phox subunit of Nox2 in ROS production in response to increased tubular flow in the mTAL of Dahl salt-sensitive rats. *Am J Physiol Renal Physiol*. 2016; 311(2):F450–8. PubMed PMID: 27279484; PubMed Central PMCID: PMC279484. DOI: 10.1152/ajprenal.00187.2016 [PubMed: 27279484]
24. Graham KA, Kulawiec M, Owens KM, Li X, Desouki MM, Chandra D, et al. NADPH oxidase 4 is an oncoprotein localized to mitochondria. *Cancer Biol Ther*. 2010; 10(3):223–31. PubMed PMID: 20523116; PubMed Central PMCID: PMC279484. [PubMed: 20523116]
25. Koziel R, Pircher H, Kratochwil M, Lener B, Hermann M, Dencher NA, et al. Mitochondrial respiratory chain complex I is inactivated by NADPH oxidase Nox4. *Biochem J*. 2013; 452(2): 231–9. PubMed PMID: 23514110. DOI: 10.1042/BJ20121778 [PubMed: 23514110]
26. Martinez-Reyes I, Diebold LP, Kong H, Schieber M, Huang H, Hensley CT, et al. TCA Cycle and Mitochondrial Membrane Potential Are Necessary for Diverse Biological Functions. *Mol Cell*. 2016; 61(2):199–209. PubMed PMID: 26725009; PubMed Central PMCID: PMC279484. DOI: 10.1016/j.molcel.2015.12.002 [PubMed: 26725009]
27. Soberanes S, Urich D, Baker CM, Burgess Z, Chiarella SE, Bell EL, et al. Mitochondrial complex III-generated oxidants activate ASK1 and JNK to induce alveolar epithelial cell death following exposure to particulate matter air pollution. *J Biol Chem*. 2009; 284(4):2176–86. PubMed PMID: 19033436; PubMed Central PMCID: PMC279484. DOI: 10.1074/jbc.M808844200 [PubMed: 19033436]
28. Muller FL, Liu Y, Van Remmen H. Complex III releases superoxide to both sides of the inner mitochondrial membrane. *J Biol Chem*. 2004; 279(47):49064–73. PubMed PMID: 15317809. DOI: 10.1074/jbc.M407715200 [PubMed: 15317809]
29. Brand MD. Mitochondrial generation of superoxide and hydrogen peroxide as the source of mitochondrial redox signaling. *Free Radic Biol Med*. 2016; 100:14–31. PubMed PMID: 27085844. DOI: 10.1016/j.freeradbiomed.2016.04.001 [PubMed: 27085844]
30. Turrens JF. Mitochondrial formation of reactive oxygen species. *J Physiol*. 2003; 552(Pt 2):335–44. PubMed PMID: 14561818; PubMed Central PMCID: PMC279484. DOI: 10.1113/jphysiol.2003.049478 [PubMed: 14561818]
31. Chen Q, Vazquez EJ, Moghaddas S, Hoppel CL, Lesnefsky EJ. Production of reactive oxygen species by mitochondria: central role of complex III. *J Biol Chem*. 2003; 278(38):36027–31. PubMed PMID: 12840017. DOI: 10.1074/jbc.M304854200 [PubMed: 12840017]
32. Kowaltowski AJ, de Souza-Pinto NC, Castilho RF, Vercesi AE. Mitochondria and reactive oxygen species. *Free Radic Biol Med*. 2009; 47(4):333–43. PubMed PMID: 19427899. DOI: 10.1016/j.freeradbiomed.2009.05.004 [PubMed: 19427899]
33. Tormos KV, Anso E, Hamanaka RB, Eisenbart J, Joseph J, Kalyanaraman B, et al. Mitochondrial complex III ROS regulate adipocyte differentiation. *Cell Metab*. 2011; 14(4):537–44. PubMed PMID: 21982713; PubMed Central PMCID: PMC279484. DOI: 10.1016/j.cmet.2011.08.007 [PubMed: 21982713]
34. Scialo F, Sriram A, Fernandez-Ayala D, Gubina N, Lohmus M, Nelson G, et al. Mitochondrial ROS Produced via Reverse Electron Transport Extend Animal Lifespan. *Cell Metab*. 2016; 23(4): 725–34. PubMed PMID: 27076081; PubMed Central PMCID: PMC279484. DOI: 10.1016/j.cmet.2016.03.009 [PubMed: 27076081]
35. Bonini MG, Consolaro ME, Hart PC, Mao M, de Abreu AL, Master AM. Redox control of enzymatic functions: The electronics of life's circuitry. *IUBMB Life*. 2014; PubMed PMID: 24668617. doi: 10.1002/iub.1258
36. Gutteridge JM, Halliwell B. Free radicals and antioxidants in the year 2000. A historical look to the future. *Ann N Y Acad Sci*. 2000; 899:136–47. PubMed PMID: 10863535. [PubMed: 10863535]
37. Miar A, Hevia D, Munoz-Cimadevilla H, Astudillo A, Velasco J, Sainz RM, et al. Manganese superoxide dismutase (SOD2/MnSOD)/catalase and SOD2/GPx1 ratios as biomarkers for tumor

- progression and metastasis in prostate, colon, and lung cancer. *Free Radic Biol Med.* 2015; 85:45–55. PubMed PMID: 25866291. DOI: 10.1016/j.freeradbiomed.2015.04.001 [PubMed: 25866291]
38. Youle RJ, Narendra DP. Mechanisms of mitophagy. *Nat Rev Mol Cell Biol.* 2011; 12(1):9–14. PubMed PMID: 21179058; PubMed Central PMCID: PMC4780047. DOI: 10.1038/nrm3028 [PubMed: 21179058]
39. Sun N, Youle RJ, Finkel T. The Mitochondrial Basis of Aging. *Mol Cell.* 2016; 61(5):654–66. PubMed PMID: 26942670; PubMed Central PMCID: PMC4779179. DOI: 10.1016/j.molcel.2016.01.028 [PubMed: 26942670]
40. Vyas S, Zaganjor E, Haigis MC. Mitochondria and Cancer. *Cell.* 2016; 166(3):555–66. PubMed PMID: 27471965; PubMed Central PMCID: PMC4503699. DOI: 10.1016/j.cell.2016.07.002 [PubMed: 27471965]
41. Schrepfer E, Scorrano L. Mitofusins, from Mitochondria to Metabolism. *Mol Cell.* 2016; 61(5):683–94. PubMed PMID: 26942673. DOI: 10.1016/j.molcel.2016.02.022 [PubMed: 26942673]
42. He C, Hart PC, Germain D, Bonini MG. SOD2 and the Mitochondrial UPR: Partners Regulating Cellular Phenotypic Transitions. *Trends Biochem Sci.* 2016; 41(7):568–77. PubMed PMID: 27180143; PubMed Central PMCID: PMC4930371. DOI: 10.1016/j.tibs.2016.04.004 [PubMed: 27180143]
43. Sullivan LB, Chandel NS. Mitochondrial reactive oxygen species and cancer. *Cancer Metab.* 2014; 2:17. PubMed PMID: 25671107; PubMed Central PMCID: PMC4323058. doi: 10.1186/2049-3002-2-17 [PubMed: 25671107]
44. Woo DK, Green PD, Santos JH, D'Souza AD, Walther Z, Martin WD, et al. Mitochondrial genome instability and ROS enhance intestinal tumorigenesis in APC(Min/+) mice. *Am J Pathol.* 2012; 180(1):24–31. PubMed PMID: 22056359; PubMed Central PMCID: PMC3338350. DOI: 10.1016/j.ajpath.2011.10.003 [PubMed: 22056359]
45. Santos JH, Meyer JN, Van Houten B. Mitochondrial localization of telomerase as a determinant for hydrogen peroxide-induced mitochondrial DNA damage and apoptosis. *Hum Mol Genet.* 2006; 15(11):1757–68. PubMed PMID: 16613901. DOI: 10.1093/hmg/ddl098 [PubMed: 16613901]
46. Singh KK. Mitochondria damage checkpoint, aging, and cancer. *Ann N Y Acad Sci.* 2006; 1067:182–90. PubMed PMID: 16803984. DOI: 10.1196/annals.1354.022 [PubMed: 16803984]
47. Hashimoto Y, Niikura T, Ito Y, Sudo H, Hata M, Arakawa E, et al. Detailed characterization of neuroprotection by a rescue factor humanin against various Alzheimer's disease-relevant insults. *J Neurosci.* 2001; 21(23):9235–45. PubMed PMID: 11717357. [PubMed: 11717357]
48. Guo B, Zhai D, Cabezas E, Welsh K, Nouraini S, Satterthwait AC, et al. Humanin peptide suppresses apoptosis by interfering with Bax activation. *Nature.* 2003; 423(6938):456–61. PubMed PMID: 12732850. DOI: 10.1038/nature01627 [PubMed: 12732850]
49. Lee C, Zeng J, Drew BG, Sallam T, Martin-Montalvo A, Wan J, et al. The mitochondrial-derived peptide MOTS-c promotes metabolic homeostasis and reduces obesity and insulin resistance. *Cell Metab.* 2015; 21(3):443–54. PubMed PMID: 25738459; PubMed Central PMCID: PMC4350682. DOI: 10.1016/j.cmet.2015.02.009 [PubMed: 25738459]
50. Chandel NS. Mitochondrial regulation of oxygen sensing. *Adv Exp Med Biol.* 2010; 661:339–54. PubMed PMID: 20204741. DOI: 10.1007/978-1-60761-500-2_22 [PubMed: 20204741]
51. Keith B, Johnson RS, Simon MC. HIF1alpha and HIF2alpha: sibling rivalry in hypoxic tumour growth and progression. *Nat Rev Cancer.* 2011; 12(1):9–22. PubMed PMID: 22169972; PubMed Central PMCID: PMC3401912. DOI: 10.1038/nrc3183 [PubMed: 22169972]
52. Semenza GL. HIF-1 and mechanisms of hypoxia sensing. *Curr Opin Cell Biol.* 2001; 13(2):167–71. PubMed PMID: 11248550. [PubMed: 11248550]
53. Li RC, Lee SK, Pouranfar F, Brittian KR, Clair HB, Row BW, et al. Hypoxia differentially regulates the expression of neuroglobin and cytoglobin in rat brain. *Brain Res.* 2006; 1096(1):173–9. PubMed PMID: 16750520. DOI: 10.1016/j.brainres.2006.04.063 [PubMed: 16750520]
54. Keith B, Simon MC. Hypoxia-inducible factors, stem cells, and cancer. *Cell.* 2007; 129(3):465–72. PubMed PMID: 17482542; PubMed Central PMCID: PMC3150586. DOI: 10.1016/j.cell.2007.04.019 [PubMed: 17482542]

55. Mazumdar J, Dondeti V, Simon MC. Hypoxia-inducible factors in stem cells and cancer. *J Cell Mol Med*. 2009; 13(11–12):4319–28. PubMed PMID: 19900215; PubMed Central PMCID: PMCPMC2874971. DOI: 10.1111/j.1582-4934.2009.00963.x [PubMed: 19900215]
56. Koh MY, Darnay BG, Powis G. Hypoxia-associated factor, a novel E3-ubiquitin ligase, binds and ubiquitinates hypoxia-inducible factor 1alpha, leading to its oxygen-independent degradation. *Mol Cell Biol*. 2008; 28(23):7081–95. PubMed PMID: 18838541; PubMed Central PMCID: PMCPMC2593390. DOI: 10.1128/MCB.00773-08 [PubMed: 18838541]
57. Chang Q, Chen B, Thakur C, Lu Y, Chen F. Arsenic-induced sub-lethal stress reprograms human bronchial epithelial cells to CD61 cancer stem cells. *Oncotarget*. 2014; 5(5):1290–303. PubMed PMID: 24675390; PubMed Central PMCID: PMCPMC4012730. DOI: 10.18632/oncotarget.1789 [PubMed: 24675390]
58. Son YO, Wang L, Poyil P, Budhraj A, Hitron JA, Zhang Z, et al. Cadmium induces carcinogenesis in BEAS-2B cells through ROS-dependent activation of PI3K/AKT/GSK-3beta/beta-catenin signaling. *Toxicol Appl Pharmacol*. 2012; 264(2):153–60. PubMed PMID: 22884995; PubMed Central PMCID: PMCPMC3462234. DOI: 10.1016/j.taap.2012.07.028 [PubMed: 22884995]
59. Huang CF, Liu SH, Lin-Shiau SY. Pyrrolidine dithiocarbamate augments Hg(2+)-mediated induction of macrophage cell death via oxidative stress-induced apoptosis and necrosis signaling pathways. *Toxicol Lett*. 2012; 214(1):33–45. PubMed PMID: 22909951. DOI: 10.1016/j.toxlet.2012.08.006 [PubMed: 22909951]
60. Lee CU, Hahne G, Hanske J, Bange T, Bier D, Rademacher C, et al. Redox Modulation of PTEN Phosphatase Activity by Hydrogen Peroxide and Bis(oxido)vanadium Complexes. *Angew Chem Int Ed Engl*. 2015; 54(46):13796–800. PubMed PMID: 26418532; PubMed Central PMCID: PMCPMC4648047. DOI: 10.1002/anie.201506338 [PubMed: 26418532]
61. Sage AP, Minatel BC, Ng KW, Stewart GL, Dummer TJ, Lam WL, et al. Oncogenomic disruptions in arsenic-induced carcinogenesis. *Oncotarget*. 2017; PubMed PMID: 28179585. doi: 10.18632/oncotarget.15106
62. Zhang F, Paramasivam M, Cai Q, Dai X, Wang P, Lin K, et al. Arsenite binds to the RING finger domains of RNF20-RNF40 histone E3 ubiquitin ligase and inhibits DNA double-strand break repair. *J Am Chem Soc*. 2014; 136(37):12884–7. PubMed PMID: 25170678; PubMed Central PMCID: PMCPMC4183597. DOI: 10.1021/ja507863d [PubMed: 25170678]
63. Son YO, Hitron JA, Wang X, Chang Q, Pan J, Zhang Z, et al. Cr(VI) induces mitochondrial-mediated and caspase-dependent apoptosis through reactive oxygen species-mediated p53 activation in JB6 Cl41 cells. *Toxicol Appl Pharmacol*. 2010; 245(2):226–35. PubMed PMID: 20298709; PubMed Central PMCID: PMCPMC5051966. DOI: 10.1016/j.taap.2010.03.004 [PubMed: 20298709]
64. Delalande O, Desvaux H, Godat E, Valleix A, Junot C, Labarre J, et al. Cadmium-glutathione solution structures provide new insights into heavy metal detoxification. *FEBS J*. 2010; 277(24):5086–96. PubMed PMID: 21078121. DOI: 10.1111/j.1742-4658.2010.07913.x [PubMed: 21078121]
65. Zhang L, Mulrooney SB, Leung AF, Zeng Y, Ko BB, Hausinger RP, et al. Inhibition of urease by bismuth(III): implications for the mechanism of action of bismuth drugs. *Biometals*. 2006; 19(5):503–11. PubMed PMID: 16937256. DOI: 10.1007/s10534-005-5449-0 [PubMed: 16937256]
66. Rikans LE, Yamano T. Mechanisms of cadmium-mediated acute hepatotoxicity. *J Biochem Mol Toxicol*. 2000; 14(2):110–7. PubMed PMID: 10630425. [PubMed: 10630425]
67. Matts RL, Schatz JR, Hurst R, Kagen R. Toxic heavy metal ions activate the heme-regulated eukaryotic initiation factor-2 alpha kinase by inhibiting the capacity of hemin-supplemented reticulocyte lysates to reduce disulfide bonds. *J Biol Chem*. 1991; 266(19):12695–702. PubMed PMID: 1676400. [PubMed: 1676400]
68. Splittgerber AG, Tappel AL. Inhibition of glutathione peroxidase by cadmium and other metal ions. *Arch Biochem Biophys*. 1979; 197(2):534–42. PubMed PMID: 507828. [PubMed: 507828]
69. Rollin-Genetet F, Berthomieu C, Davin AH, Quemeneur E. Escherichia coli thioredoxin inhibition by cadmium: two mutually exclusive binding sites involving Cys32 and Asp26. *Eur J Biochem*. 2004; 271(7):1299–309. PubMed PMID: 15030480. DOI: 10.1111/j.1432-1033.2004.04037.x [PubMed: 15030480]

70. Sisombath NS, Jalilehvand F. Similarities between N-Acetylcysteine and Glutathione in Binding to Lead(II) Ions. *Chem Res Toxicol*. 2015; 28(12):2313–24. PubMed PMID: 26624959; PubMed Central PMCID: PMC4688085. DOI: 10.1021/acs.chemrestox.5b00323 [PubMed: 26624959]
71. Myers JM, Antholine WE, Myers CR. Hexavalent chromium causes the oxidation of thioredoxin in human bronchial epithelial cells. *Toxicology*. 2008; 246(2–3):222–33. PubMed PMID: 18328613; PubMed Central PMCID: PMC4688085. DOI: 10.1016/j.tox.2008.01.017 [PubMed: 18328613]
72. Carvalho CM, Chew EH, Hashemy SI, Lu J, Holmgren A. Inhibition of the human thioredoxin system. A molecular mechanism of mercury toxicity. *J Biol Chem*. 2008; 283(18):11913–23. PubMed PMID: 18321861. DOI: 10.1074/jbc.M710133200 [PubMed: 18321861]
73. Myers CR. The effects of chromium(VI) on the thioredoxin system: implications for redox regulation. *Free Radic Biol Med*. 2012; 52(10):2091–107. PubMed PMID: 22542445; PubMed Central PMCID: PMC4688085. DOI: 10.1016/j.freeradbiomed.2012.03.013 [PubMed: 22542445]
74. Kalinowski DS, Stefani C, Toyokuni S, Ganz T, Anderson GJ, Subramaniam NV, et al. Redox cycling metals: Pedaling their roles in metabolism and their use in the development of novel therapeutics. *Biochim Biophys Acta*. 2016; 1863(4):727–48. PubMed PMID: 26844773. DOI: 10.1016/j.bbamcr.2016.01.026 [PubMed: 26844773]
75. Tao R, Coleman MC, Pennington JD, Ozden O, Park SH, Jiang H, et al. Sirt3-mediated deacetylation of evolutionarily conserved lysine 122 regulates MnSOD activity in response to stress. *Mol Cell*. 2010; 40(6):893–904. PubMed PMID: 21172655; PubMed Central PMCID: PMC4688085. DOI: 10.1016/j.molcel.2010.12.013 [PubMed: 21172655]
76. Ozden O, Park SH, Kim HS, Jiang H, Coleman MC, Spitz DR, et al. Acetylation of MnSOD directs enzymatic activity responding to cellular nutrient status or oxidative stress. *Aging (Albany NY)*. 2011; 3(2):102–7. PubMed PMID: 21386137; PubMed Central PMCID: PMC4688085. DOI: 10.18632/aging.100291 [PubMed: 21386137]
77. Padmaja Divya S, Pratheeshkumar P, Son YO, Vinod Roy R, Andrew Hitron J, Kim D, et al. Arsenic Induces Insulin Resistance in Mouse Adipocytes and Myotubes Via Oxidative Stress-Regulated Mitochondrial Sirt3-FOXO3a Signaling Pathway. *Toxicol Sci*. 2015; 146(2):290–300. PubMed PMID: 25979314. DOI: 10.1093/toxsci/kfv089 [PubMed: 25979314]
78. Lombard DB, Alt FW, Cheng HL, Bunkenborg J, Streeper RS, Mostoslavsky R, et al. Mammalian Sir2 homolog SIRT3 regulates global mitochondrial lysine acetylation. *Mol Cell Biol*. 2007; 27(24):8807–14. PubMed PMID: 17923681; PubMed Central PMCID: PMC4688085. DOI: 10.1128/MCB.01636-07 [PubMed: 17923681]
79. van der Vliet A, Janssen-Heininger YM. Hydrogen peroxide as a damage signal in tissue injury and inflammation: murderer, mediator, or messenger? *J Cell Biochem*. 2014; 115(3):427–35. PubMed PMID: 24122865; PubMed Central PMCID: PMC4688085. DOI: 10.1002/jcb.24683 [PubMed: 24122865]
80. Forman HJ, Torres M. Redox signaling in macrophages. *Mol Aspects Med*. 2001; 22(4–5):189–216. PubMed PMID: 11679166. [PubMed: 11679166]
81. Zhou R, Tardivel A, Thorens B, Choi I, Tschopp J. Thioredoxin-interacting protein links oxidative stress to inflammasome activation. *Nat Immunol*. 2010; 11(2):136–40. PubMed PMID: 20023662. DOI: 10.1038/ni.1831 [PubMed: 20023662]
82. Baig MS, Zaichick SV, Mao M, de Abreu AL, Bakhshi FR, Hart PC, et al. NOS1-derived nitric oxide promotes NF-kappaB transcriptional activity through inhibition of suppressor of cytokine signaling-1. *J Exp Med*. 2015; 212(10):1725–38. PubMed PMID: 26324446; PubMed Central PMCID: PMC4688085. DOI: 10.1084/jem.20140654 [PubMed: 26324446]
83. Strebovsky J, Walker P, Lang R, Dalpke AH. Suppressor of cytokine signaling 1 (SOCS1) limits NFkappaB signaling by decreasing p65 stability within the cell nucleus. *FASEB J*. 2011; 25(3):863–74. PubMed PMID: 21084693. DOI: 10.1096/fj.10-170597 [PubMed: 21084693]

Figure 1.
Schematic Representation of some of the molecular pathways by which heavy metals and arsenic cations amplify oxidative stress promoting disease permissive cellular phenotypes.