

Class 1: The high price of energy: Mitochondrial production of ROS

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 Sep 19;278 (38):36027- 31.

Take home message: A majority of mitochondrial ROS are produced from complex I and complex III. Cytosolic ROS is most likely to come from Complex III because the Q cycle allows for superoxide release into the intermembrane space whereas Complex I ROS remain in the lumen where they can be detoxified by antioxidant enzymes.

Campian JL, Qian M, Gao X, Eaton JW. Oxygen tolerance and coupling of mitochondrial electron transport. J Biol Chem. 2004 Nov 5;279(45):46580-7

Take home message: If somehow mitochondrial transfer could be coupled with higher efficiency to oxygen ability in all cells, ROS production would not be an issue. As it is, 1-4% of all oxygen consumed gets converted to ROS.

RESOURCE for: Mitochondrial inhibitors, ROS measurements

Class 2: Radical messengers: ROS as facilitators of cellular signaling (ROS participate in directed, controlled physiological functions under normal circumstances)

Meng TC, Fukada T, Tonks NK. Reversible oxidation and inactivation of protein tyrosine phosphatases (PTPs) in vivo. Mol Cell. 2002 Feb;9(2):387-99.

Take home message: All PTPs have a conserved active site cysteine that needs to remain reduced for catalytic activity. Therefore, increased intracellular ROS lead to more downstream kinase-induced phosphorylation activity. Theory: This mechanism evolved to allow for a temporal and spatial “lag” to prevent wasteful cycles of dephosphorylation and phosphorylation at G-protein receptors.

Kamata H, Honda S, Maeda S, Chang L, Hirata H, Karin M. Reactive oxygen species promote TNF α -induced death and sustained JNK activation by inhibiting MAP kinase phosphatases. Cell. 2005 Mar 11;120(5):649-61.

Take home message: ROS are agents of programmed cell death by mediating the signal transduction cascade mediated by the major tumor necrosis factor cytokine TNF α .

RESOURCE for: Novel protocol for assessing dephosphorylation activity by a given phosphatase, overview of JNK signaling pathways.

Class 3: Hired assassins: ROS in anti-pathogen defense

Kim YS, Morgan MJ, Choksi S, Liu ZG. TNF-induced activation of the Nox1 NADPH oxidase and its role in the induction of necrotic cell death. Mol Cell. 2007 Jun 8;26(5):675-87.

Take home message: ROS are not just byproducts of faulty respiration, specific enzymes (NADPH oxidases, NOX) exist in the cells to produce them in controlled amounts. ROS are required for sustained kinase-based signaling.

Helmerhorst EJ, Troxler RF, Oppenheim FG. The human salivary peptide histatin 5 exerts its antifungal activity through the formation of reactive oxygen species. Proc Natl Acad Sci U S A. 2001 Dec 4;98(25):14637-42

Take home message: An example of how ROS act directly in antifungal activity against a common form of yeast infections. Discusses a chemical mimetic for SOD activity.

RESOURCE for: Overview of NOXs and programmed immune as well as direct-damage induced cell death in anti-pathogen defense.

Class 4: Antioxidants: Fighting the good fight

Jang M, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CW, Fong HH, Farnsworth NR, Kinghorn AD, Mehta RG, Moon RC, Pezzuto JM. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science. 1997 Jan 10;275 (5297):218-20.

Take home message: Epigallocatechin gallates (EGCG - found in Snapple, red wine and many teas) like resveratrol are one of the few dietary antioxidants which have been rigorously shown to have some benefit in alleviating a cancer risk as well as insulin-signaling based syndromes in animal models.

Serafini M, Bugianesi R, Maiani G, Valtuena S, De Santis S, Crozier A. Plasma antioxidants from chocolate. Nature. 2003 Aug 28; 424(6952):1013

Take home message: Milk tends to antagonize the antioxidant effects of chocolates and other polyphenolic antioxidants.

RESOURCE for: Cool facts you can throw out at parties over a dark chocolate truffle or a glass of wine!

Class 5: The free radical theory: ROS and aging (The most commonly accepted and studied theory of aging)

Trifunovic A, Hansson A, Wredenberg A, Rovio AT, Dufour E, Khvorostov I, Spelbrink JN, Wibom R, Jacobs HT, Larsson NG. Somatic mtDNA mutations cause aging phenotypes without affecting reactive oxygen species production. Proc Natl Acad Sci U S A. 2005 Dec 13;102(50):17993-8.

Take home message: Suggests aging-associated ROS may lie upstream of mitochondrial mutations not downstream.

Something to think about: What causes ROS to be produced in sufficient quantities to initiate the process of aging? What is the threshold sensor for ROS?

Schriner SE, Linford NJ, Martin GM, Treuting P, Ogburn CE, Emond M, Coskun PE, Ladiges W, Wolf N, Van Remmen H, Wallace DC, Rabinovitch PS. Extension of murine life span by overexpression of catalase targeted to mitochondria. Science. 2005 Jun 24; 308(5730):1909-11

Take home message: One of several animal models which demonstrates that reducing ROS levels can alleviate the aging phenotype.

RESOURCE for: Understanding how transgenic mouse models are used for research.

Class 6: The root of the problem: oxidative damage in stem cell renewal

Ito K, Hirao A, Arai F, Takubo K, Matsuoka S, Miyamoto K, Ohmura M, Naka K, Hosokawa K, Ikeda Y, Suda T. Reactive oxygen species act through p38 MAPK to limit the lifespan of hematopoietic stem cells. Nat Med. 2006 Apr;12(4):446-51.

Take home message: At least in the hematopoietic system, stem cells may be more vulnerable to oxidative stress and damage than more differentiated cells

Nijnik A, Woodbine L, Marchetti C, Dawson S, Lambe T, Liu C, Rodrigues NP, Crockford TL, Cabuy E, Vindigni A, Enver T, Bell JI, Slijepcevic P, Goodnow CC, Jeggo PA, Cornall RJ. DNA repair is limiting for haematopoietic stem cells during ageing. Nature. 2007 Jun 7;447(7145):686-90.

Take home message: Oxidative damage becomes important in reducing stem cell regeneration capacity in animal models as they age. An answer to what lies at the root of how ROS induce aging in whole organisms?

RESOURCE for: Understanding the hematopoietic stem cell system and how they are characterized experimentally, an introduction to DNA repair systems

Class 7: Balancing act: ROS effects on insulin resistance and diabetes

Houstis, N., Rosen, ED, Lander, ES. Reactive oxygen species have a causal role in multiple forms of insulin resistance. Nature 2006 Apr 13;440 (7086):944-8.

McClung JP, Roneker CA, Mu W, Lisk DJ, Langlais P, Liu F, Lei XG. Development of insulin resistance and obesity in mice overexpressing cellular glutathione peroxidase. Proc Natl Acad Sci U S A. 2004 Jun 15;101(24):8852-7

Take home message collectively: ROS are major effectors of insulin resistance a symptom of Type II and a side-effect of Type I diabetes. However therapies that seek to modulate ROS levels need to take account of the physiological functions of ROS required for normal cellular signaling.

RESOURCE for: An introduction to unbiased microarray screening to find novel target for disease therapy.

Class 8: Breaking hearts: ROS in ischemic reperfusion injury

Zweier JL, Flaherty JT, Weisfeldt ML. Direct measurement of free radical generation following reperfusion of ischemic myocardium. Proc Natl Acad Sci U S A. 1987 Mar;84(5):1404-7.

Take home message: Introduction to Electron Paramagnetic Resonance - a way to detect ROS in living systems. Direct evidence that ischemic reperfusion produces ROS.

Dewald O, Frangogiannis NG, Zoerlein M, Duerr GD, Klemm C, Knuefermann P, Taffet G, Michael LH, Crapo JD, Welz A, Entman ML. Development of murine ischemic cardiomyopathy is associated with a transient inflammatory reaction and depends on reactive oxygen species. Proc Natl Acad Sci U S A. 2003 Mar 4;100(5):2700-5.

Take home message: Demonstration of above in an animal model.

RESOURCE for: Understanding the most common source of cardiac injury and possible therapeutic avenues, EPR.

Class 9: Brain drain: oxidative stress in neurodegenerative diseases

Lu T, Pan Y, Kao SY, Li C, Kohane I, Chan J, Yankner BA. Gene regulation and DNA damage in the ageing human brain. *Nature*. 2004 Jun 24;429(6994):883-91.
Bruijn LI, Houseweart MK, Kato S, Anderson KL, Anderson SD, Ohama E, Reaume AG, Scott

Take home message: Oxidative DNA damage doesn't have to be random and can affect transcription by preferentially damaging promoter. Cu^+ is a powerful Fenton reactive agent.

RW, Cleveland DW. Aggregation and motor neuron toxicity of an ALS-linked SOD1 mutant independent from wild-type SOD1. *Science*. 1998 Sep 18;281(5384):1851-4

Take home message: Although ALS is caused by a mutation in an antioxidant protein, the causative disease pathology may have nothing to do with ROS but with the aberrant protein structures made by the mutant protein.

RESOURCE For: Overview of ROS in neurodegeneration, understanding systems of oxidative DNA damage and repair and how cause-and-effect experiments are critical to understanding whether ROS are upstream or downstream of a physiological phenomenon.

Class 10: Foot-soldiers of renegade cells: ROS in cancer and oncogenic transformation

Dolado I, Swat A, Ajenjo N, De Vita G, Cuadrado A, Nebreda AR. p38alpha MAP kinase as a sensor of reactive oxygen species in tumorigenesis.

Cancer Cell. 2007 Feb;11(2):191-205

Take home message : Many common oncogenes produce oxidative stress in one form or the other. An elevation in cellular oxidative stress may be an important part of the cell's early warning system that it is on its way to becoming cancerous. Successful cancers can disable this warning system by decoupling the ROS sensing ability of the stress-responsive proteins.

Radisky DC, Levy DD, Littlepage LE, Liu H, Nelson CM, Fata JE, Leake D, Godden EL, Albertson DG, Nieto MA, Werb Z, Bissell MJ. Rac1b and reactive oxygen species mediate MMP-3-induced EMT and genomic instability.

Nature. 2005 Jul 7;436(7047):123-7

Take home message: The processes that operate in embryonic development get re-activated in invasive tumors and one of the major pathways is modulated by ROS levels.

RESOURCE for: Understanding the major oncogenes, basic cancer biology techniques, the process of invasion and metastasis.

Class 11: Fighting fire with fire: more ROS or less ROS as therapeutic strategies?

Ohsawa I, Ishikawa M, Takahashi K, Watanabe M, Nishimaki K, Yamagata K, Katsura K, Katayama Y, Asoh S, Ohta S. Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals. Nat Med. 2007 Jun;13(6):688-94.

Take home message: An animal model of therapy for ischemic reperfusion injury in the brain that preferentially targets the hydroxyl radical (most damaging ROS) and can be easily delivered to most tissues without side-effects.

Trachootham D, Zhou Y, Zhang H, Demizu Y, Chen Z, Pelicano H, Chiao PJ, Achanta G, Arlinghaus RB, Liu J, Huang P. Selective killing of oncogenically transformed cells through a ROS-mediated mechanism by beta-phenylethyl isothiocyanate. Cancer Cell. 2006 Sep;10 (3):241-52.

Take home message: For cancer therapy, the answer may lie in increasing rather than decreasing ROS effects since tumor cells typically have higher oxidative stress than normal cells to begin with.

Priya's Parting Shots

- Many different intra- and extracellular stresses function through ROS - IR radiation, oncogenes, inflammation inducers, several major chemotherapeutic drugs etc.
- Every experiment should have a positive control (push the issue artificially and show you get the desired result; this is especially true if the result you wish to show is a negative outcome of some experimental protocol) and a negative control (do a mock treatment and show you don't get the same result; this is particularly true if you see a positive outcome across the board).
- Experiments analyzing oxidative stress in different samples or systems must be conducted in exactly the same way and normalized to some common baseline before they can be extrapolated to each other. *Remember the isogenic cell line experiments are more convincing than measuring ROS levels in random cancer cells vs. random normal cells.
- Hydrogen peroxide and superoxide are NOT intrinsically damaging species; they need to feed into the Fenton reaction: H_2O_2 (also produced by catalytic or spontaneous superoxide dismutation) + Fe^{2+} (released from metalloproteins by superoxide) $\rightarrow \text{OH}\cdot$ the most damaging ROS and one that has no endogenous detoxifying system.
- Several indicators of oxidative stress should correlate quantitatively before a particular system or treatment can be concluded to produce ROS effects - either signaling or damage ie. remember that if protein carbonyls are going up then so should DNA damage markers.