Control of RNA oxidation as a novel mechanism for preventing mitochondrial dysfunction

Sulochan Malla, Merritt Walker and Zhongwei Li
Department of Biomedical Science, Florida Atlantic University, Boca Raton, FL 33471

Background

Mitochondria generate energy through oxidative phosphorylation in eukaryotic cell and produce large amount of reactive oxygen species (ROS) as byproducts during this process. In particular in mitochondria, oxidative modifications of biomolecules by ROS can cause their inactivation. The situation is exacerbated during oxidative stress when excessive amounts of ROS are produced. Oxidative damage of macromolecules causes mitochondrial dysfunction and eventually leads to numerous diseases such as cardiovascular and neural disorders. Although the deleterious effects of oxidized DNA, proteins and lipids have been extensively characterized, little is known about the potential causative effects of oxidized RNA.

Here, we assessed RNA oxidation levels in the mitochondria and cytosol of cultured human cells and examine if RNA plays role in making mitochondria dysfunctional. We previously showed that human polynucleotide phosphorylase (hPNPase), which mainly localizes to mitochondria and binds oxidized RNA with high affinity, reduces RNA oxidation and protects HeLa cell during oxidative stress[1]. We intend to elucidate the potential role of hPNPase and its associated RNA helicase, hSUV3[2, 3], in reducing mtRNA oxidation.

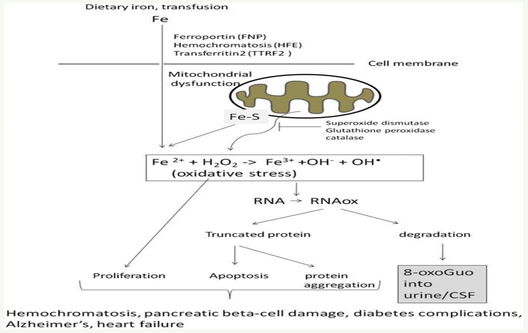


Fig.1 Poulson et al 2012.Free radical biology and medicine 52(2012) 1352-1361

Objectives

1. **Investigate the rate of RNA oxidation during oxidative stress conditions and compare the level inside mitochondria and cytosol.**

2. **Examine the effect of mitochondrial enzymes on modulating the oxidation level of RNA.**

Experimental methodology

Human cells (HLEB3/HeLa)
↓
siRNA/hPMP knockdown/overexpression of hPNP
↓
With or Without H_2O_2
↓
Total mitochondrial and cytosol RNA
↓
P1 Digestion
↓
HPLC

8-oxo-Guanosine (CalBiochem) analysis

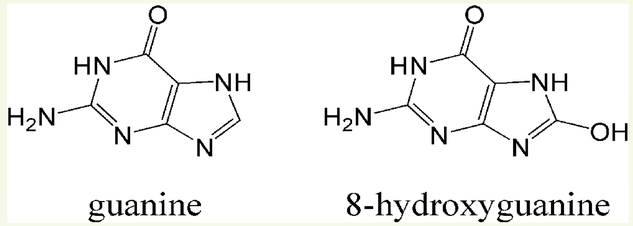


Fig 2. The structure of guanine and 8-hydroxyguanine (drawn by ChemDraw Ultra 8.0).

Results

1. Effect of oxidative stress on RNA oxidation level.

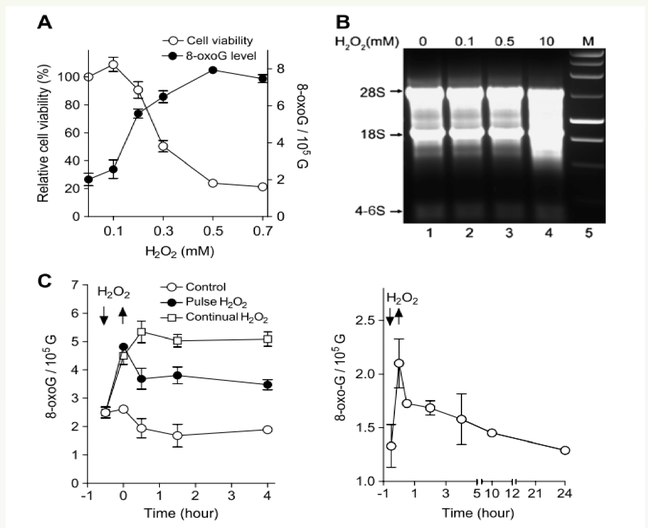
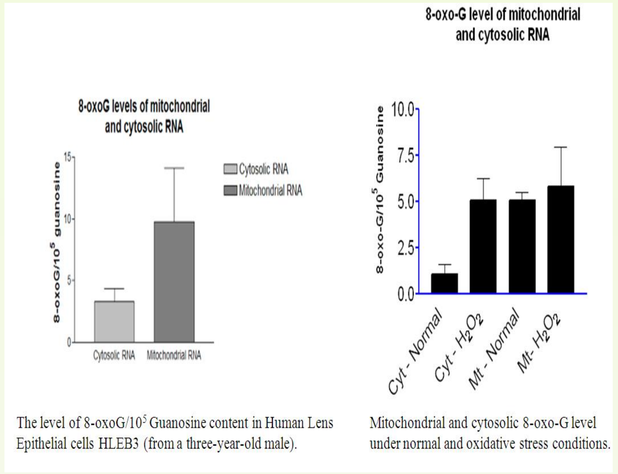


Fig 4A. Inverse relationship between RNA oxidation level (8-oxo-G) and oxidative stress. B. Fragmentation of different rRNA types. C .Confirmation of Fig A by pulse treatment

Results

2. Comparative study of mtRNA and cytosolic RNA in HLEB3 and HeLa cells under normal and oxidative stress condition



The level of 8-oxoG/10^5 Guanosine content in Human Lens Epithelial cells HLEB3 (from a three-year-old male).

Mitochondrial and cytosolic 8-oxo-G level under normal and oxidative stress conditions.

Fig 3.Right: Level of 8-oxo-Guanosine in cytosolic and mitochondrial fraction from HLEB3 cell line and Left: HeLa cells under normal and oxidative stress conditions. Mitochondrial and cytosolic 8-oxo-G level under normal and oxidative stress conditions

3. Role of hPNPse in reducing the level of oxidized RNA

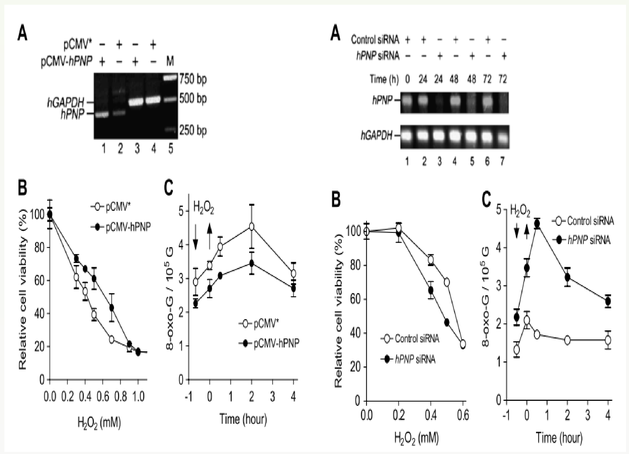
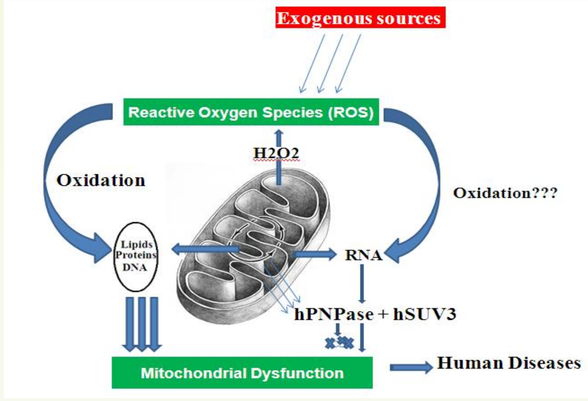


Fig 5A. Left, over expression of hPNPase and B & C Role of hPNPase cell viability and 8-oxo-G level. Fig A. right, knock down of hPNP and B & C . Role of hPNPase in reducing RNA oxidation and HeLa cell viability

Conclusion

1. RNA are oxidized to a greater extent during oxidative stress.
2. MtRNA are oxidized more than cytosolic RNA in both Human Lens Epithelial cells and HeLa cells under normal and oxidative stress conditions.
3. hPNpase has been shown to play an important role in reducing the level of oxidized RNA

Future Directions



References

1. Wu, J., & Li, Z. (2008). Biochemical and Biophysical Research Communications, 372(2), 288–92. doi:10.1016/j.bbrc.2008.05.058
2. Borowski, L. S., Dziembloowski, A., Hejnowicz, M. S., Stepien, P. P., & Szczesny, R. J. (2013). Nucleic Acids Research, 41(2), 1223–40. doi:10.1093/nar/gks1130
3. Chujo, T., Ohira, T., Sakaguchi, Y., Goshima, N., Nomura, N., Nagao, A., & Suzuki, T. (2012). Nucleic Acids Research, 40(16), 8033–47. doi10.1093/nar/gks506

Acknowledgement

Dr. Mark Kantorow and Danial Chauss
NIH grant GMO97693 to Dr. Z.LI
Florida Atlantic University Healthy Ageing Research Initiative Seed Grant to Dr. Zhongwei Li.