Changes in Mitochondrial Respiration during Cell Senescence

Sebastian A. Vasquez Lopez - BSc (Hons) Genetics - 106003204
Email: s.a.vasquez-lopez@newcastle ac.uk

Institute for Ageing and Health

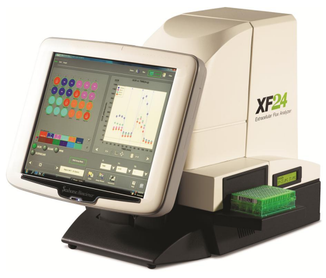
1. Introduction

Recent studies on human fibroblasts showed that production of reactive oxygen species (ROS), mitochondrial mass and oxygen consumption increase during replicative cell senescence[1].

These changes are also known to appear in DNA damage induced senescence after X-ray irradiation:

- The peak in ROS production occurs post 2 days
- The levels remain high for at least 10 days

In order to study how these changes co-relate with **mitochondrial respiration** over time, bioenergetics experiments were performed using the **Seahorse Bioscience XF24 analyzer**[2].

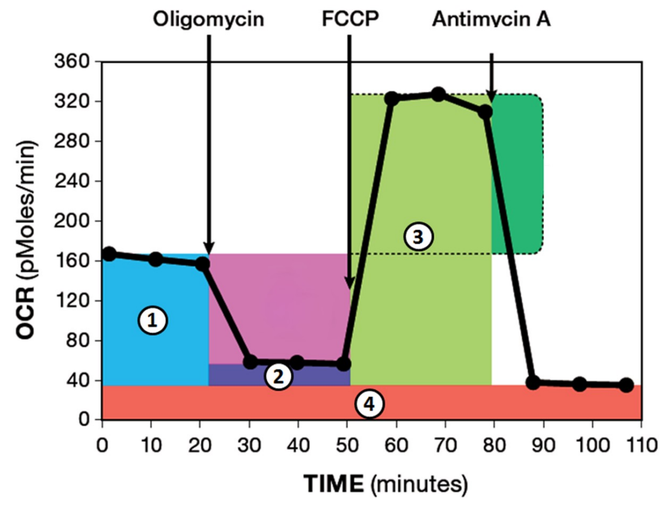


2. Methods

Young MRC5 human fibroblasts were seeded in Seahorse XF24 plates, X-irradiated with 20Gy and kept for: **1 day, 2 days, 7 days** and **14 days**. Two independent repeats of the series were carried out.

At each time-point measurements of the oxygen consumption rates of the irradiated cells were performed, at four different respiratory states:

1. Basal (minus non-mitochondrial)
2. Proton leak (treated with the ATPase inhibitor **oligomycin**)
3. Uncoupled (treated with the uncoupling agent **FCCP**)
4. Non-mitochondrial (treated with the complex III inhibitor **antimycin**)



After each run the raw data was corrected by the actual cell number per well and the respiratory parameters per cell calculated.

3. Results

Proton leak, **basal** and **maximum respiration** *increased* significantly at 7 days after irradiation and the levels remain high for at least 14 days. Respiration rates in replicative senescent cells resembled those of 14 days post irradiation (***Figure 1***).

The increase in proton leak and mitochondrial respiration appeared *later*, than the increase in mitochondrial mass and ROS production i.e. **7 days** vs. **2 days** (***Figure 2***). Respiration and especially proton leak kept increasing for longer, with post 14-day rates more than six times higher than those of young cells (*Figure 1*).

The response of cells to pyruvate along with other parameters of mitochondrial function calculated from the respiratory data stayed relatively constant in induced and replicative senescence (data not shown).

Figure 1. **Relative increase in mitochondrial respiration during induced and replicative senescence**

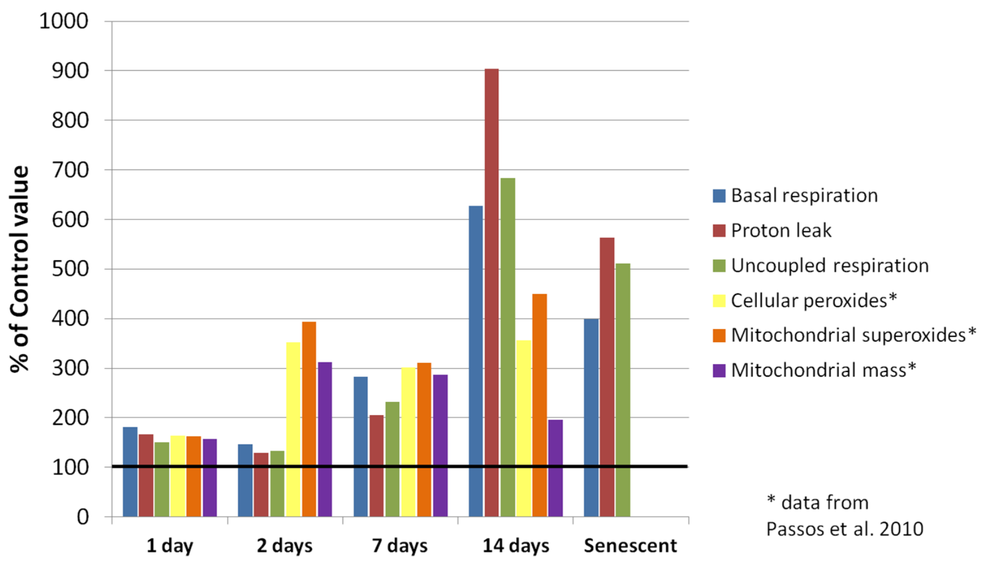
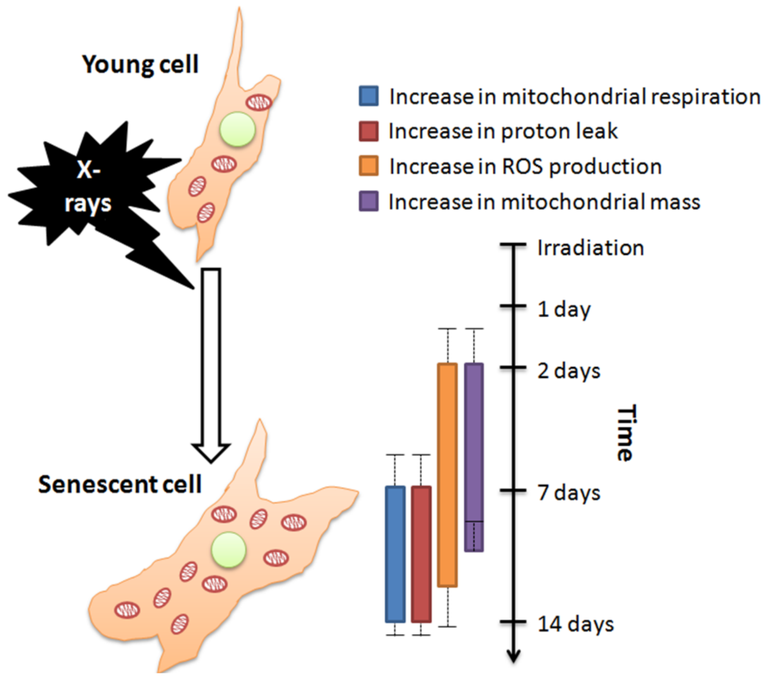


Figure 2. **Time of appearance of mitochondrial changes during induced senescence**



4. Conclusions

- These findings suggest that the increase in oxygen consumption might be brought about, at least in some extent, by mechanisms other than increased mitochondrial mass and ROS production.
- The capacity of mitochondria to increase respiration upon stimulation does not appear to be compromised in senescent cells which contradicts previously reported findings using high-resolution respirometry[1].
- Further experiments should assess whether the inhibition of the DNA damage response – ROS feedback loop[1] can reverse or prevent the bioenergetic changes reported here.

References

1. Passos, J.F. et al., 2010. Feedback between p21 and reactive oxygen production is necessary for cell senescence. Molecular systems biology, 6(347), 347
2. Gerencser, A. a et al., 2009. Quantitative microplate-based respirometry with correction for oxygen diffusion. Analytical chemistry, 81(16), 6868