

MITOEAGLE WG4 Cell Lines

Discussion: Considerations when measuring, and “comparing and contrasting” oxygen consumption in cultured cells (cultured primary cells, stem cells, other cell lines)

1. Is there a PRIMARY CELL REFERENCE? *e.g.* primary hepatocytes to compare with HepG2’s.

2. PASSAGE NUMBER:

Does cellular mitochondrial oxygen consumption wane with increased passage number?

(a) primary cell cultures, *e.g.* maximum passage number 7/8 in sertoli cells

(b) Cell lines, relationship with passage number not too well studied

Eveline HUTTER , Kathrin RENNER, Gerald PFISTER, Petra STOCKL, Pidder JANSEN-DÜRR and Erich GNAIGER:

Senescence-associated changes in respiration and oxidative phosphorylation in primary human fibroblasts. *Biochem. J.* (2004) 380, 919–928

3. ASSESSING BASELINE PARAMETERS BEFORE ASSESSING CELLULAR OXYGEN CONSUMPTION

(a) Is division symmetrical?

(b) What is the doubling time?

(c) Assess cell-cycle profile.

(d) Markers of cell type/stem cell type (Q-RTPCR, FACS, enzymes)

(e) Karyotyping

4. MEDIUM to be USED

- (a) Medium used *e.g.* DMEM, RPMI etc
- (b) Glucose concentration is regularly a major variable
- (c) Are fatty acids in the medium/to be added to the medium? saturated/unsaturated
- (d) Stem cells, using KSR with/instead for FBS?

5. STP v NORMOXIA v HYPOXIA

Consideration should be given to growth or at least treatment/analysis at conditions equivalent to those *in vivo e.g.* Normoxia to minimise ROS damage

6. ASSESSMENT of OXYGEN CONSUMPTION not due to OXIDATIVE PHOSPHORYLATION

Usually more in cell lines

- (a) NOS
- (b) NOX
- (c) XO
- (d) HO (Nrf2)
- (e) MAO
- (f) CytochromeP450 related enzymes
- (g) Peroxisomal (catalase)

7. CULTURE TYPE

(a) 2D

(b) 3D matrix cancer constructs: perhaps diffusion limiting for direct oxygen consumption measurements but fluorescent markers of bioenergetics function could be used to get a in vitro bioenergetic profile of a solid tumour

8. QUESTIONS at the round table discussion yesterday

(i) Cell permeabilization was the main focus

ACTIONS

9. Review article(s) acting as a database and reference article comparing and contrasting bioenergetics studies

The review(s) should contain

- (i) cancer cell lines (taking into consideration source type, differentiation state, phenotypic state *etc*)
- (ii) Stem cells (IPS, adult stem cells, embryonic)
- (iii) Primary cell cultures
- (iv) Reference to freshly isolated primary cell data where possible
- (v) Attempts to compare published data in a standardised comparable format
- (vi) Recommendation for a standard terminology to be used when working with cultured cells (from WG1)

10. Division of labour for cultured cells for which there may be significant oxygen consumption data:

Hepatocytes:- Jordi Muntané, Roberto Scatena other volunteers

Breast Cancers: Roberto Scatena, Richie Porter, other volunteers

Kidney cells: Andras Meszaros, other volunteers

Sertoli cells: Luís Crisostomo, Dias, Raquel Bernardino, other volunteers

Stem cells: Raj Rao, Shilpa Iyer, Guida Bento, other volunteers

Muscle cells *e.g.* C₂C₁₂ cells Kasja Pavlovic, Erich Gnaiger, volunteers needed

Fibroblasts: Pidder Jansen-Dürr, Erich Gnaiger, other volunteers

Bone marrow derived macrophages: volunteers needed

Lung: Porter, other volunteers needed

Other cells types: Cancer stem cells (Roberto Scatena), cardiomyoblasts (Luciana Ferreira)