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-DÙRR 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 is 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 Scantena other volunteers
Breast Cancers: Roberto Scantena, Richie Porter, other volunteers
Kidney cells: Andras Meszaros, other volunteers
Sertoli cells: Luís Crisostomo, Dias, Bernardino, other volunteers
Stem cells: Raj Rao, Shilpa Iyer, Guida Bento,, other volunteers
Muscle cells *e.g.* C_2C_{12} cells Kasja Pavlovic, Erich Gnaiger, volunteers needed
Fibroblasts: Pidder Jensen-Dürr, Erich Gnaiger, other volunteers
Bone marrow derived macrophages: volunteers needed
Lung: Porter, other volunteers needed
Other cells types: Cancer stem cells (Roberto Scantena), cardiomyoblasts (Luciana Ferreira)