Interrogating gynaecological cancer cell metabolism at different oxygen tensions: Results using a novel modified atmosphere cellular handling system

Ayşe Latif [1], Jason Bruce [4], Evan Kitsell [3], Fergus Murray [3], Emma Crosbie [2], Ian J. Stratford [1]

1 Experimental Oncology Group, Manchester Pharmacy School, Faculty of Medical and Human Sciences, Stopford Building, Oxford Road, M13 9PL.
2 Women's Cancer Centre, Institute of Cancer Sciences, University of Manchester, St Mary's Hospital, Oxford Road, Manchester, M13 9WL.
3 Don Whitley Scientific Limited, 14 Otley Road, Shipley, West Yorkshire, BD17 7SE.
4 Channels and Transporters Research Group, Faculty of Life Sciences, University of Manchester, Michael Smith Building, Oxford Road, M13 9PL.

Introduction

According to Cancer Research UK, around 200,000 new cases of gynaecological cancers are diagnosed in Europe every year. Potentially 75% of these cases are cancer types that could benefit from improved treatment regimes. A recent collaborative project between the University of Manchester and Don Whitley Scientific is contributing significant research in this fight against gynaecological cancer.

Gynaecological cancer cells demonstrate increased rates of glycolysis and lactate production. These traits have been suggested to predict an increased likelihood of metastasis, resistance to therapy and reduced survival in affected individuals. Lactate transport in cancer cells is carried out by members of the monocarboxylate transporter (MCT) family, notably MCT1 and 4. Thus, we hypothesized that pharmacologic inhibition of MCTs could improve treatment outcome by reducing the glycolytic potential of these tumour cells.

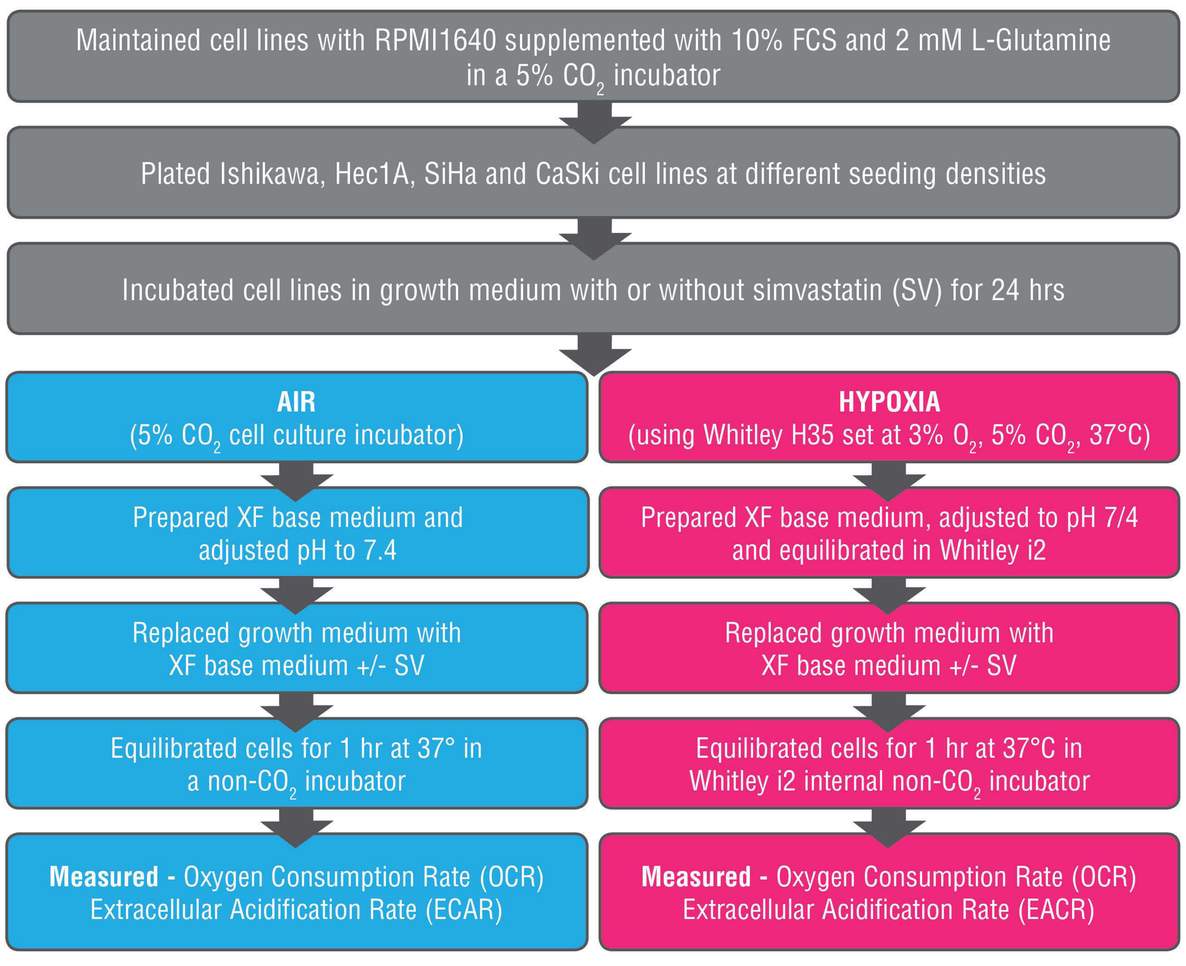
Our initial work has comprised of metabolic profiling of endometrial (Ishikawa and Hec1A) and cervical (SiHa and CaSki) cancer cell lines in air by measuring response to glycolytic and mitochondrial stress test compounds using a Seahorse Bioscience XF^{e}96 Extracellular Flux Analyzer. Furthermore, the metabolism of Ishikawa cell lines was measured in air and hypoxia with or without 24 hour simvastatin (potential MCT inhibitor) treatment.

A novel workstation, comprising two chambers linked by a transfer tunnel, allows the use of the Seahorse metabolic analyzer under variable oxygen tensions and thus provides a unique platform for metabolic analysis of cell lines in hypoxia as well as air.

With this equipment, cell lines are prepared in the first chamber (Whitley H35 Hypoxystation) under user selectable hypoxic conditions and then transferred (without exposure to ambient laboratory conditions) into a second chamber (Whitley i2 Workstation), purpose-designed to accommodate a Seahorse Bioscience XF^{e}96 Extracellular Flux Analyser, operating under different oxygenation conditions, as specified by Seahorse Bioscience.

The system incorporates several new and novel features.

Methods



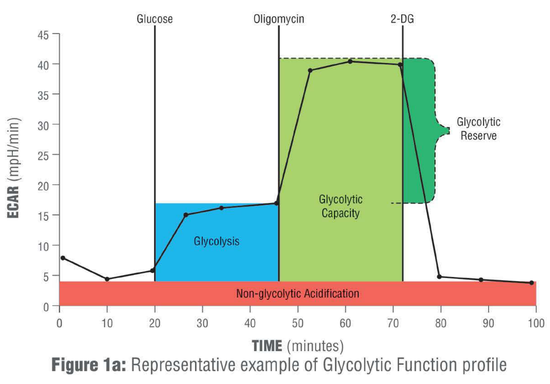


Figure 1a: Representative example of Glycolytic Function profile

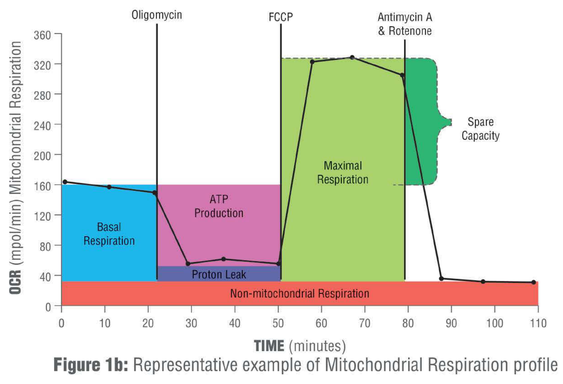


Figure 1b: Representative example of Mitochondrial Respiration profile

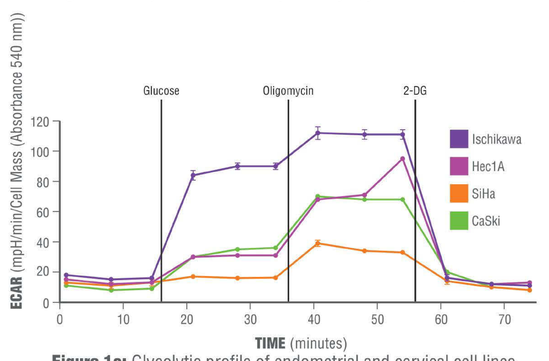


Figure 1c: Glycolytic profile of endometrial and cervical cell lines

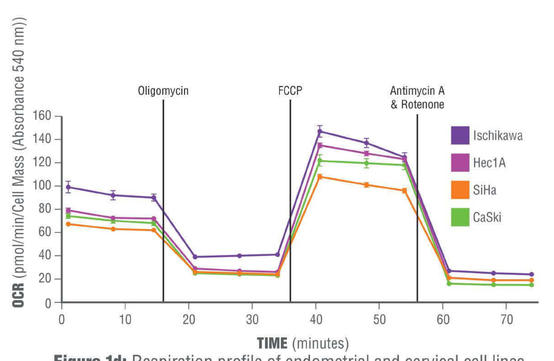


Figure 1d: Respiration profile of endometrial and cervical cell lines

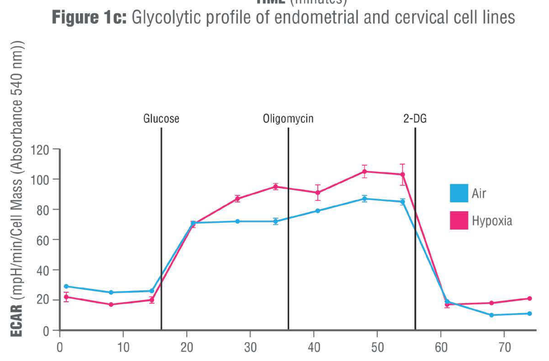


Figure 1e: Glycolytic profile of Ishikawa cells in air and hypoxia

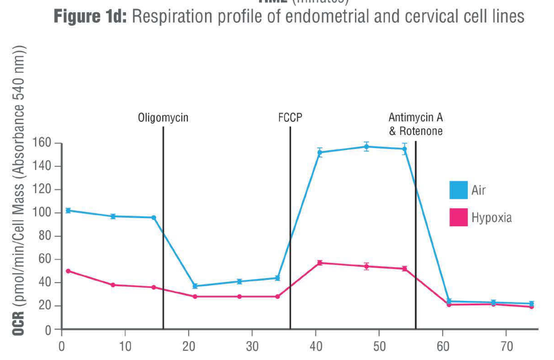


Figure 1f: Respiration profile of Ishikawa cells in air and hypoxia

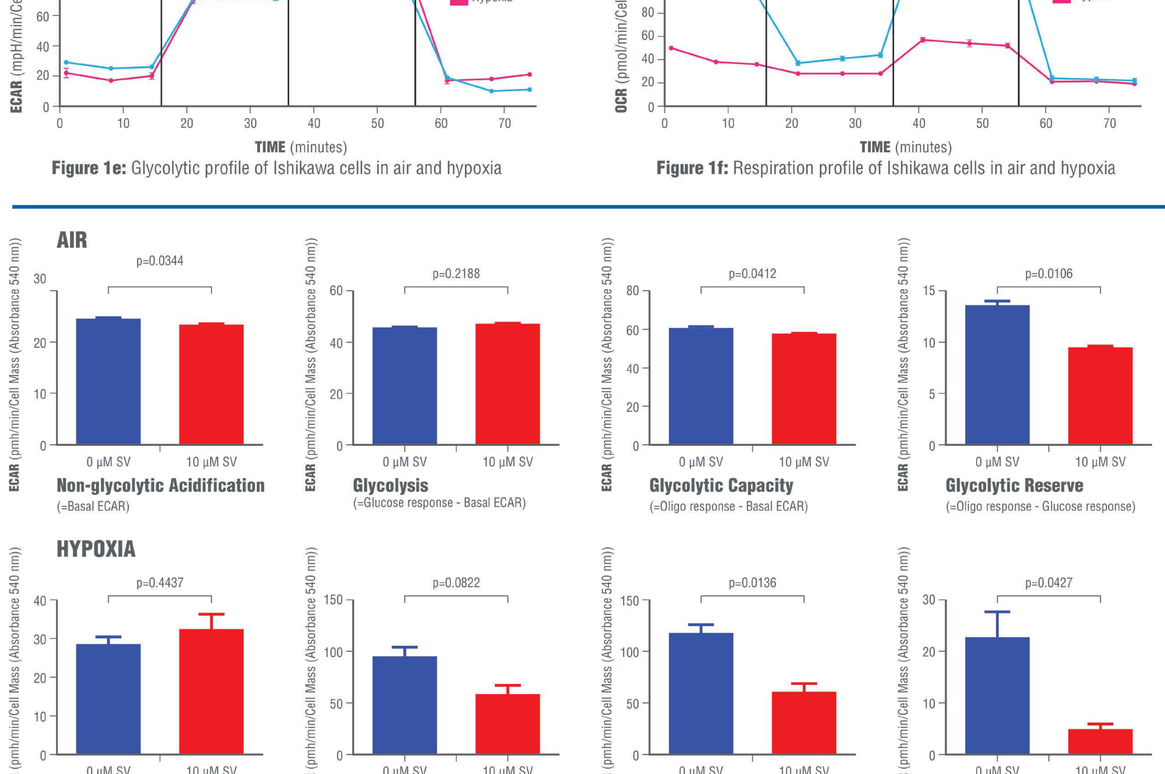


Figure1g: The effect of 24 Hours 10 µM simvastatin (SV) treatment on Ishikawa glycolysis in air and hypoxia

Figure1h: The effect of 24 Hours 10 µM simvastatin (SV) treatment on Ishikawa respiration in air and hypoxia

Acknowledgement: This project is funded by the National Institute for Health Research, Research and Innovation Division, Strategic Project Funding.

Results

The metabolic profiles of endometrial and cervical cell lines differ from each other:

- Ishikawa cell line utilizes glycolysis for energy production and has more glycolytic capacity than CaSki, SiHa and Hec1A cell lines (Fig 1c).
- Endometrial and cervical cell lines utilise respiration for energy production at slightly different levels (Fig 1d).

The metabolic profile of Ishikawa cell line in air and hypoxia differs as follows:

- Ishikawa cell line increases glycolysis and its glycolytic capacity under hypoxic conditions (Fig 1e).
- Respiratory profile of Ishikawa cell line dramatically reduced under hypoxic conditions (Fig 1f).

Treatment of 10 µM SV for 24 hours significantly reduced:

- glycolytic capacity and the reserve of Ishikawa cell lines in air and hypoxia (Fig 1g).
- basal respiration, maximal respiration and respiratory capacity in air and in hypoxia (Fig 1h).
- non-mitochondrial respiration in hypoxia (Fig 1h).

Conclusions / Future Direction

We have demonstrated the following:

- The combination of a Whitley H35 HEPA Hypoxystation and a Whitley i2 Instrument Workstation (Don Whitley Scientific) provides a suitable hypoxic environment in which a Seahorse Bioscience XF^{e}96 Extracellular Flux Analyzer can be used to measure cell metabolism at 3% oxygen concentration.
- Simvastatin treatment has an impact on glycolysis and could contribute to reports showing simvastatin may be beneficial for the treatment of a variety of cancers.

Our future experiments will focus on:

- Determining hypoxia response in the remaining three cell lines with or without simvastatin treatment.
- Determining the lowest oxygen level in which cell metabolism can be successfully measured with a Seahorse XF^{e}96 Analyzer.

Whitley i2 Instrument Workstation connected to a Whitley H35 HEPA Hypoxystation

The Whitley i2 Instrument Workstation provides a controlled environment as defined by Seahorse Bioscience, in which to house their Extracellular Flux Analyzers.

Seahorse Bioscience XF^{e}96 Extracellular Flux Analyzer
Seahorse Analyzers measure oxygen consumption and extracellular acidification rate in real-time.

Poster first shown at 1st Seville Molecular Medicine Workshop, Seville, Spain. 1-2 December 2014.
Data generated by University of Manchester. Poster produced by Graphic Design Department, Don Whitley Scientific Limited.

www.dwscientific.co.uk