Application Note

Bioenergetic analysis of suspension cells: hematopoietic stem cells and lymphocytes

A real-time assay that quantifies the ATP and biosynthetic demands of immune cell proliferation, differentiation and effector function.

RESEARCH AREAS

Immunology
Cancer
Translational Medicine

ASSAY TYPE

ATP and biosynthetic demand: Assessing respiration and glycolysis in suspension cells

KEYWORDS

Hematopoietic stem cells, Lymphocytes, leukemia cells, Cell-Tak™, mitochondrial respiration, glycolysis, mitochondrial function

Normal suspension cells, such as hematopoietic cells and lymphocytes are responsible for supplying oxygen to the body and protecting the host. Cellular metabolism is markedly dynamic in immune responses during proliferation and immune effector functions.[1,2] For example, antigen stimulated T cells display a metabolic switch to aerobic glycolysis accompanied by cell growth and proliferation, similar to the Warburg effect observed in cancer cells. Manipulation of the lymphocyte-specific metabolic control pathways may prove useful in treating diseases characterized by immune hyperactivation, including leukemia and autoimmune disorders.

Immobilization of non-adherent cells in XF cell culture microplates enables bioenergetic analysis of lymphocytes and hematopoietic cells with the XF Extracellular Flux Analyzer. Immobilization can be achieved by using microplates coated with Cell-Tak™ Adhesive, a formulation of non-immunogenic, polyphenolic proteins extracted from the marine mussel.

In a recent publication, Cappasso, *et al.*[3] investigated the role of the voltage-gated proton channel HVCN1 in cellular metabolism of B lymphocytes activation using B cells immobilized with Cell-Tak. Bioenergetic analyses of the B cells were generated by measuring the oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) of B cells as indications of mitochondrial respiration and glycolysis, respectively. The authors assessed mitochondrial respiration and glycolysis in B cells activated for 24 hours with F(ab')2 anti-IgM. The B cell receptor (BCR) activated B cells experienced an increase in both mitochondrial respiration and glycolysis when compared to non-stimulated B cells. (Figure 1)

The authors then showed that genetic deletion of HVCN1 significantly impaired the increase in metabolism of activated B cells, but not that of resting B cells. These impairments result from disrupted signal transduction of the BCR complex and downstream signaling of Akt activation. It was determined that

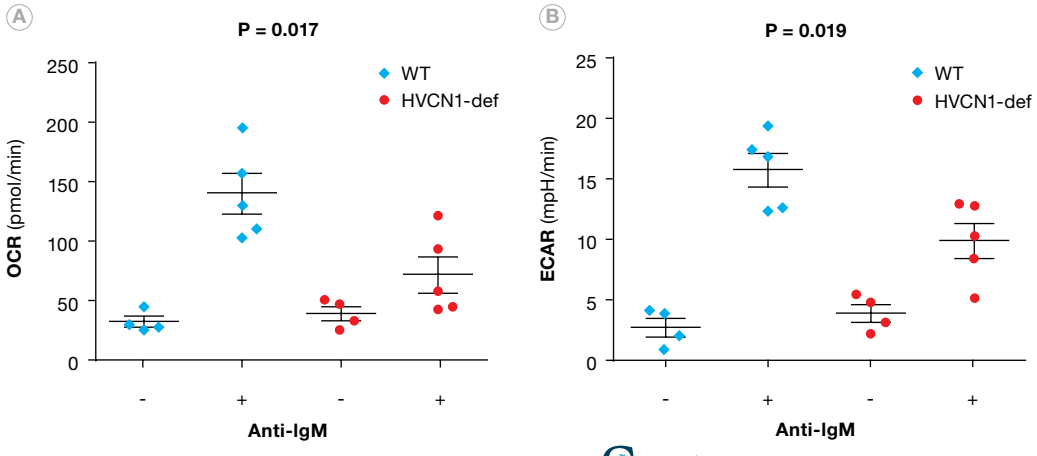


Figure 1 | HVCN1 ion channel deficiency in B cells results in impaired cellular metabolism

Metabolic rates in wild-type and HVCN1-deficient B cells before (–) and after (+) stimulation for 24 h with F(ab')2 anti-IgM (20 µg/ml), presented as the oxygen consumption rate (OCR) and extracellular acidification rate (ECAR), measures of mitochondrial respiration and glycolysis, respectively. Each symbol represents an individual mouse; longer horizontal lines indicate the mean, and small horizontal lines indicate the standard error. P values, Student's T-test. Data are representative of three experiments with four mice (time 0) or five mice (24 hours).

Seahorse Bioscience
A part of **Agilent Technologies**

after BCR stimulation, cells lacking HVCN1 displayed lower oxygen consumption and acidification than did wild-type cells (Figure 1), which indicated impaired energy production via mitochondrial oxidative phosphorylation and glycolysis in the absence of HVCN1 in the activated B cells.

Consistent with a defect in cellular metabolism, HVCN1-deficient B cells showed less proliferation *in vitro* after stimulation with F(ab')2 anti-IgM and defective antibody response *in vivo*.

Discussion

Immobilization of suspension cells makes it possible to monitor mitochondrial respiration and glycolysis of leukocytes in the XF Analyzer, and to connect cellular energy metabolism to cellular function, for example, immune responses or dysfunction such as leukemia.

Liu *et al*[4] demonstrated that polycomb repressor Bmi1 has an important role in maintaining mitochondrial function and redox homeostasis of thymocytes and hematopoietic stem cells. The authors analyzed the mitochondrial function of freshly isolated Bmi1-/- murine thymocytes. They showed that intact Bmi1-/- thymocytes had both reduced basal mitochondrial oxygen consumption and reduced mitochondrial oxidative capacity. Consistent with these results, the authors found significant impairment in the function of isolated Bmi1-/- mitochondria including reduced electron flow, increased NAD(P)H levels, and increased MitoSox staining, supporting their hypothesis that mitochondria are the major source of increased ROS level in Bmi1-/- thymocytes.

Abnormal metabolism is a hallmark of many types of cancers including leukemia. In another study, Wu *et al.*[5] investigated the effect of the anticancer drug Imatinib, which targets the Bcr-Abl oncogene, on Bcr-abl-expressing K562 leukemia cells. It was found that K562 cells treated with Imatinib for 48 hours reduced mitochondrial respiration and glycolysis, which is accompanied by a decreased cell proliferation rate. These results suggest that Imanitib's anticancer effect is mediated, at least in part, through altering cellular energy metabolism.

Most recently, Gurumurthy *et al.*[6] demonstrated that Lkb1 tumor suppressor is critical for the maintenance of energy homeostasis in haematopoietic cells, independent of AMP-activated protein kinase (AMPK), and mammalian target of rapamycin (mTOR) signaling. It was found that Lkb1-deficient bone marrow cells exhibit mitochondrial defects with markedly reduced mitochondrial respiratory capacity, among the other metabolic defects. These results define a central role for Lkb1 in broadly maintaining energy homeostasis in haematopoietic cells through a novel metabolic checkpoint.

One concern about assaying immune cells such as B and T lymphocytes, immobilized on culture surface, is the potential for cell activation by the crosslinking of the cells during immobilization. This is unlikely for at least two reasons. Firstly, the immobilized cells are analyzed within a very short time period, typically two hours, which precedes the surface expression of early activation marker CD26[6]. Secondly, as shown in Figure 1, it is evident that stimulated and un-stimulated B cells showed a clear bioenergetic difference suggesting the naïve cells are not activated by the immobilization. Poly-D-lysine (another nonspecific attachment factor) treated plates, have also been used successfully to immobilize lymphocytes for XF bioenergetic assay.

Immobilization of suspension cells makes it possible to monitor mitochondrial respiration and glycolysis of leukocytes in the XF Analyzer, and to connect cellular energy metabolism to cellular function, for example, immune responses or dysfunction such as leukemia.

Materials and Methods

Cells and Compounds: XF V7 cell culture microplates were coated with Cell-Tak™ (BD Bioscience) prior to plating B cells as described in the Seahorse protocol.

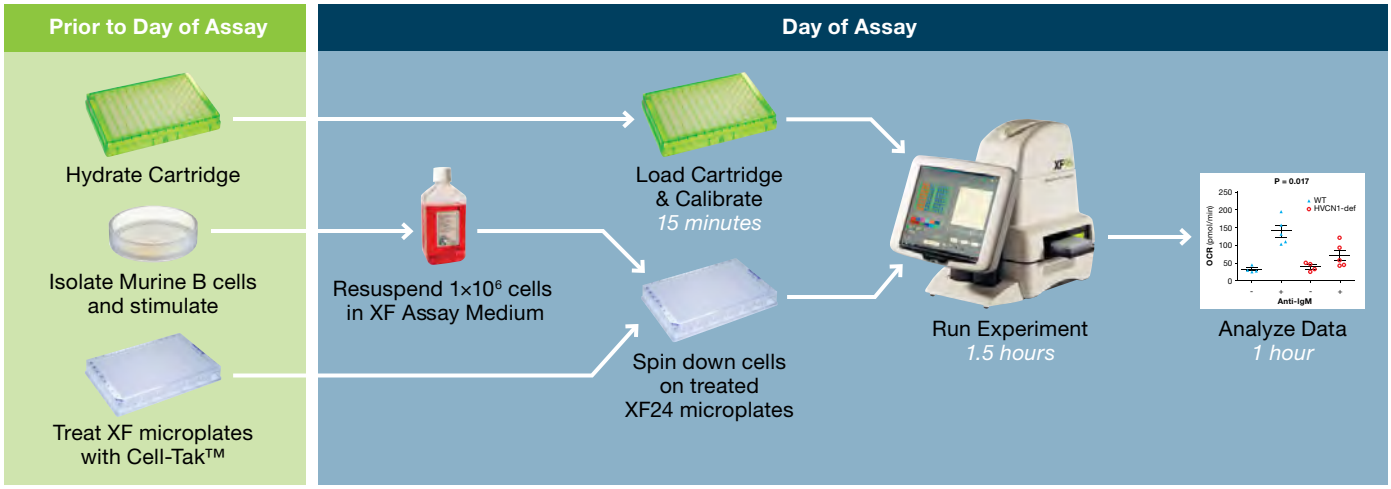
B cells were purified from spleens of 8- to 12-week-old mice either by negative selection with anti-CD43 magnetic beads, with a purity of ~95%, or by positive selection with anti-B220 and anti-CD19 magnetic beads (Miltenyi Biotech), with a purity of ~98%. Cells were cultured in RPMI complete medium containing 10% (vol/vol) FCS, penicillin and streptomycin, l-Glutamax and 50 µM 2-mercaptoethanol. Splenic B cells were stimulated for various times at 37 °C with goat anti–mouse IgM F(ab')2 fragment (20 µg/ml). They were stimulated for 24 hours with F(ab')2 anti-IgM (20 µg/ml). Stimulated or control B cells were resuspanded in bicarbonate-free and low buffered DMEM (pH 7.4) containing 11 mM glucose, 2 mM glutamine and 1 mM pyruvate.

XF Bioenergetic Analysis

Bioenergetic analyses of lymphocytes were performed in the XF Analyzer (Seahorse Bioscience). The XF Analyzer creates a transient micro-chamber of only a few microliters in specialized cell culture microplates. This enables OCR (oxygen consumption rate) and ECAR (extracellular acidification rate) to be monitored in real time.[7,8]

Wild-type and HVCN1 deficient B cells stimulated for 24 hours were plated in Cell-Tak™ coated 24-well XF V7 cell culture microplate at 1×10^6 cells per well in 100 µL assay medium. The cells were allowed to become attached for 30 minutes in a 37°C non-CO_2 incubator. 500 µL assay medium was added to each well after the cells were stably attached to the bottom of the wells and incubated for one additional hour prior to XF bioenergetic assay. Low buffered bicarbonate-free DMEM assay medium contains 11 mM glucose, 2 mM glutamine and 1 mM pyruvate. Four basal OCR and ECAR of the control and stimulated cells were measured, and the average of two basal rates of each sample were plotted (Figure 1).

Figure 2 | Flow Chart of XF Assay



References

1. Jones RG, Thompson CB: *Revving the engine: signal transduction fuels T cell activation*. Immunity 2007, 27:173-178.
2. Pearce EL. *Metabolism in T cell activation and differentiation*. Curr Opin Immunol. 2010 22(3):314-20.
3. Capasso M, et al. *HVCN1 modulates BCR signal strength via regulation of BCR-dependent generation of reactive oxygen species*. Nat Immunol. 2010. 11(3):265-72.
4. Liu J, et al. *Bmi1 regulates mitochondrial function and the DNA damage response pathway*. Nature. 2009. 459(7245):387-92.
5. Wu M, et al. *A Novel Technology for Profiling Energy Metabolism in Cancer Cells*. Poster presented at GTCbio 2nd Symposium on Tumor Growth and Tumor Progression. 2005. Boston, MA.
6. Gurumurthy S, et al. *The Lkb1 metabolic sensor maintains haematopoietic stem cell survival*. Nature. 2010. 468(7324):659-63.
7. Simms PE, Ellis TM. *Utility of flow cytometric detection of CD69 expression as a rapid method for determining poly- and oligoclonal lymphocyte activation*. Clin Diagn Lab Immunol. 1996. 3(3):301-4.
8. Wu M, et al. *Multiparameter metabolic analysis reveals a close link between attenuated mitochondrial bioenergetic function and enhanced glycolysis dependency in human tumor cells*. Am J Physiol Cell Physiol. 2007 292(1):C125-36.
9. Ferrick DA et al. *Advances in measuring cellular bioenergetics using extracellular flux*. Drug Discov Today. 2008. 13(5-6):268-74.

Corporate Headquarters

Seahorse Bioscience Inc.
16 Esquire Road
North Billerica, MA 01862 US
Phone: 1.978.671.1600

European Headquarters

Seahorse Bioscience Europe
Fruebjergvej 3
2100 Copenhagen DK
Phone: +45 31 36 98 78

Asia-Pacific Headquarters

Seahorse Bioscience Asia
199 Guo Shou Jing Road, Suite 207
Pudong, Shanghai 201203 CN
Phone: 0086 21 33901768

www.seahorsebio.com

Seahorse *Bioscience*

A part of **Agilent Technologies**

201112302