Agilent Case Study

Optimizing CAR T Cell Development and Function Using Quantitative Metabolic Data

Introduction

Recent studies have found that cellular metabolic properties can influence chimeric antigen receptor (CAR) T cell antitumor function and power CAR T cell longevity and persistence in the tumor microenvironment; therefore, it is important to understand CAR T cell metabolic phenotypes. Such knowledge can help researchers reprogram the metabolic pathways of CAR T cells during their engineering and production to help improve efficacy.

Michael Boateng-Antwi, PhD., a clinical researcher in the laboratory of Stephan Grupp, MD, PhD., one of the pioneers in the field of CAR-T cell therapy at the Children's Hospital of Philadelphia (CHOP), currently studies the molecular mechanisms of CAR T cell exhaustion and lentiviral vector engineering strategies to enhance the antitumor function of the cells. For his research, quantitative data are a key—and Dr. Boateng-Antwi is achieving it using the Agilent Seahorse XF Pro analyzer and the Seahorse XF T Cell Metabolic Profiling kit. "The new kit and the Pro are definitely a game changer for us," said Dr. Boateng-Antwi.

Used to assess mitochondrial and glycolytic activity simultaneously in live cells, the Seahorse XF T Cell Metabolic Profiling kit has helped CHOP researchers improve the quality of their data and focus on the most promising CAR constructs. Dr. Boateng-Antwi was introduced to the Seahorse XF Pro platform by his colleague, Roddy O'Connor, PhD., a faculty member of Pathology and Laboratory Medicine at the Perelman School of Medicine, University of Pennsylvania.

Dr. O'Connor's lab has established expertise in applying Seahorse metabolic analysis to preclinical CAR T cell research for many years. "The Seahorse is a must-have tool for us as it is very informative. It's everyone's first go-to approach," said Dr. O'Connor. Studying the metabolic parameters of T cells exposed to antigens repeatedly, his team is now mapping out cellular responses in these serial stimulation assays. The Seahorse XF Pro analyzer has become a powerful tool for these studies, generating robust and reproducible data.

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Michael Boateng-Antwi, PhD.

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The power of innovative product design

Innovative product design contributes to the power of the Seahorse XF Pro analyzer and its reagent kits. Traditional metabolic assays, such as the Cell Mito Stress Test, use carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone (FCCP) as a mitochondrial uncoupler to assess spare respiratory capacity (SRC) for metabolic research, which can lead to variable results in T cells. The Agilent Seahorse XF T Cell Metabolic Profiling kit uses 2-fluorophenyl {6-[(2-fluorophenyl) amino] (1,2,5-oxadiazolo[3,4-e] pyrazin-5-yl)} amine (BAM15) as an uncoupler to ensure accurate and reproducible measurements of SRC and maximal respiratory capacity in T cells.

Dr. O'Connor, a long-standing user of traditional Seahorse technologies, said that XF BAM15 and the Seahorse XF Pro analyzer were important technological advances for T cell research and CAR T development. "…It makes me think of the advance when we went from 24-well plates to 96-well plates—the 96-well plate analyzer was a great advance, and the Seahorse XF Pro is as good as that advance," he said.

Dr. Boateng-Antwi had been using an older instrument and the traditional assay kit with FCCP on frozen T cells in the beginning of his work at CHOP. The variable results generated by the FCCP created some problems in the beginning of the project. But after using the Seahorse XF Pro analyzer and XF T Cell Metabolic Profiling kit, he is now generating "textbook-like" data (Figure 1). "The XF BAM15, I think, has been a game-changer," Dr. Boateng-Antwi said. Dr. O'Connor added, "It was phenomenal data that was really accentuated in the resting T cells."

Dr. Boateng-Antwi also commented on the "ready-to-go" nature of the Agilent XF BAM15 in the XF T Cell Metabolic Profiling kit and its ease-of-use. "I was telling one of my undergrads that you could even close your eyes and easily reconstitute the reagents, no complex calculation, no stocks, and no messing up with dilutions," he said.

While SRC is considered a powerful attribute of cellular energy metabolism, Dr. O'Connor thinks more attention should be paid to glycolysis as well. The Seahorse T Cell Metabolic Profiling kit enables the lab to simultaneously examine the role of SRC and glycolysis in T cell persistence and fitness, potentially revealing new insights. In combination with the Agilent xCELLigence RTCA eSight assay, researchers in Dr. O'Connor's lab are using these new Seahorse tools to elucidate the role of glycolysis and how it connects to cytotoxicity. Although the exact link has yet to be demonstrated, he believes glycolysis could play a key role.

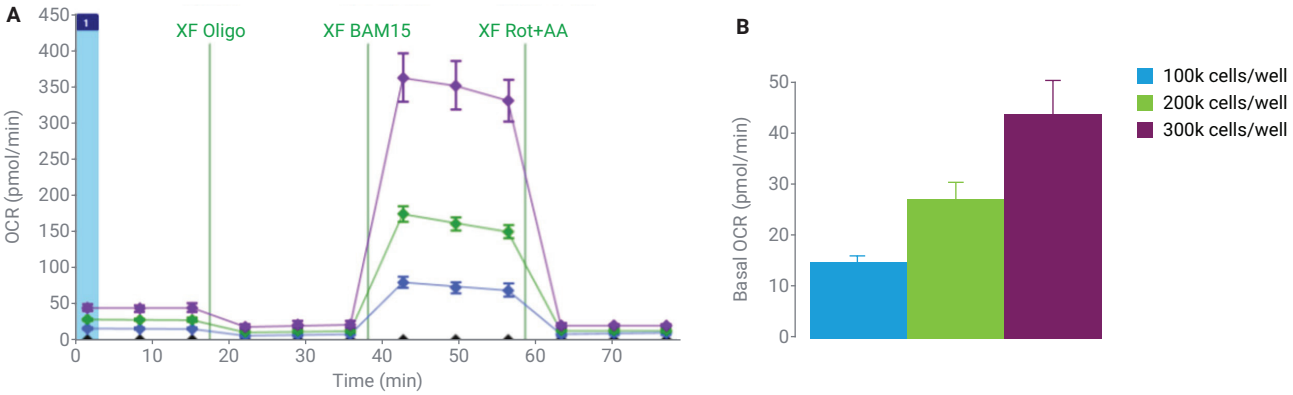

Figure 1. Cell density titration with human naïve T cells using the XF T Cell Metabolic Profiling Kit. Three replicate wells for each condition. XF RPMI pH 7.4 assay medium supplemented with 10 mM glucose, 2 mM glutamine and 1 mM pyruvate, was used. (A) Typical OCR kinetic traces showing the responses to mitochondrial modulators with high reproducibility or low error bars for 100k and 200k cells/well. (B) Basal OCR bar chart comparing the rates at different seeding densities. Optimal seeding density is determined as 200k cells/well, as it gives reasonable basal and maximal OCR values and tight error bars.

A must-have tool for metabolic studies and CAR T development

One of the primary goals of Dr. Boateng-Antwi's lab is to generate CAR T cells for mouse model infusion and study the bioenergetics of the cells over time. They rely on this information to help optimize the CAR T cell manufacturing process and determine how steps in that process impact cell persistence and exhaustion. Using the Seahorse kits on the XF Pro analyzer, the lab can generate quantitative bioenergetic data. These data enable the researchers to objectively compare the different CAR T cells and monitor the mouse models post infusion. Dr. Boateng-Antwi reports that "[Seahorse] assays have been extremely important to us, a must-have tool for us, so we can quickly remove constructs that are not helping," Dr. Boateng-Antwi said.

Dr. O'Connor's research team started investigating the metabolic properties of CAR T cells with Seahorse technology several years ago to distinguish why the 28 Zeta CAR T cells burn and die quickly, while the BB Zeta CAR T cells are more long-lasting. These studies led to the discovery that the choice of signaling domain can metabolically reprogram T cells to alter their mitochondrial function and persistence[1], providing foundational knowledge for developing metabolic strategies to improve CAR T cell therapies. "We always look at the Seahorse as very informative. It's like a biomarker almost, indicating the quality of the T cell product that we're working with," Dr. O'Connor said. The researchers in his lab are now using the Seahorse XF Pro analyzer and the T Cell Metabolic Profiling kit in their serial stimulation assays. These assays help map what happens when T cells are repeatedly exposed to antigen, a condition CAR T cells encounter post infusion, and help identify favorable metabolic phenotypes under such conditions.

An area of focus for Dr. O'Connor's lab is to improve T cell metabolic attributes through genetic engineering. Examples include introducing novel and even non-native enzymes into T cells (like what is published on *Lactobacillus brevis* NADH oxidase[2]). They were able to show that these cells have increased lactate consumption. Thus, through genetically engineered T cells, they can confer unique metabolic traits favorable for survival and function in solid tumor environments. "We did it by Seahorse. I think it's a powerful tool to determine whether we are making effective T cells," Dr. O'Connor said. He added that for everyone who has a metabolic question, it is always recommended to perform an Seahorse assay. "It's everyone's first, go-to approach—let's try the Seahorse," Dr. O'Connor said.

High sensitivity for quiescent cells and low-abundance samples

In clinical research applications, samples from in vivo sources are often in a resting state (quiescent). For these samples, it is key to have a sensitive technology to detect baseline oxygen consumption rate (OCR). The Seahorse XF Pro analyzer and the XF T Cell Metabolic Profiling kit deliver the sensitivity and robustness necessary to discern slight differences in these samples while minimizing the amount of sample required.

In Dr. Boateng-Antwi's lab, a comparison study was performed using an older technology and the Seahorse XF Pro analyzer. The researchers stimulated expanded T cells repeatedly over a period of 15 days. From the very beginning, they observed a lag in terms of picking up the signals with the older technology, compared to the XF Pro analyzer. "By day 15, you could see that the XF Pro was just phenomenal in terms of the data it was showing. For the same sample on these two machines, the XF Pro was able to pick up very sensitive data. It was definitely a game-changer," Dr. Boateng-Antwi said.

Dr. O'Connor also commented that the XF Pro analyzer would be valuable for clinical labs and any labs with clinical samples, as they are often resting cells. The analyzer is also essential for research labs that perform xenograft, collecting tumor infiltrating lymphocyte (TIL) cells. If TILs are collected from the tumor at a time when the CAR T is not working, they become hyper-functional. The XF Pro analyzer could pick up a lot more baseline OCR that could potentially be missed with older technologies, as these cells are quiescent, inert, and not metabolically active. "We need the technology to meet the biology, and I think the XF Pro and XF BAM15 do that, because the combination allows us to see differences that we wouldn't have seen," Dr. O'Connor said.

Conclusion

To help optimize CAR T cell development and manufacturing, researchers are studying the links between cellular metabolism, bioenergetics, and CAR T cell function. To produce quantitative, reliable results, sensitive technology is needed for success, especially in low-abundance samples. Leading CAR T cell researchers, Drs. O'Connor and Boateng-Antwi, have used the Seahorse XF Pro analyzer and Seahorse XF T Cell Metabolic Profiling kit to achieve robust results, gaining unique and deeper insights to understand how to improve CAR T cell antitumor function.

References

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