Application Brief

Immunotherapy

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Mitochondrial Metabolism Is a Key Indicator of CAR T Cell Therapeutic Efficacy in Chronic Lymphocytic Leukemia

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Introduction

The development of cell-mediated immunotherapies has revolutionized cancer research as well as the study of the immune system. One of the most promising types of cell therapy involves the genetic engineering of novel chimeric antigen receptor (CAR) T cells to target cancer cells. Although CAR T cell therapy has the potential to induce sustained remission in patients with refractory disease, recent trials have reported that it only does so in a fraction of patients with chronic lymphocytic leukemia (CLL).[1] The underlying reasons for these suboptimal results are currently unclear, however, one likely factor in CLL is acquired T cell dysfunction.[2-5]

There is a growing appreciation of the intricate link between T cell function and cellular metabolic strategies, underlining the value of immune cell therapies and metabolic modulation to optimize cell products for better efficacy in patients.[6-10] As illustrated in Figure 1, quiescent or resting T cells primarily meet their energy demands using mitochondrial oxidative phosphorylation (OXPHOS). Upon activation, T cells undergo rapid metabolic reprogramming and increase their dependence on glycolysis and overall energy production. This switch enables them to quickly meet the enhanced energetic demands required for effector function.[9] Measuring the metabolic phenotype provides an opportunity to modulate metabolic pathways and tune metabolic processes for enhanced immune cell performance.

Glycolytic impairments in activated $CD8^+$ T cells from CLL patients have been demonstrated in previous studies.[11] This application brief reviews a study by Jaco van Bruggen and colleagues, which set out to establish whether CLL cells also impair mitochondrial function, due to the importance of OXPHOS in the early events of T cell activation.[5]

Study results

Using Agilent Seahorse XF technology, Jaco van Bruggen *et al.* demonstrated that CLL-derived CD8⁺ T cells exhibit a skewed metabolic profile associated with reduced mitochondrial fitness. Comparing to healthy donor derived CD8⁺ T cells, resting CD8⁺ T cells in CLL exhibit elevated oxygen consumption rate (OCR). They also showed that this increase in OXPHOS could not be attributed to increases in mitochondrial mass (Figure 1).[5]

In addition, CLL derived CD8⁺ T cells showed reduced spare respiratory capacity (SRC) when compared to those from healthy donors.[5] SRC is a validated and reliable measure of mitochondrial function and is well established as a strong indicator of immune cell fate and fitness.[7,12] This reduction in SRC indicates that CLL-derived CD8⁺ T cells have a reduced capacity to respond to changes in bioenergetic demands, such as those involved in T cell activation.

van Bruggen and colleagues also questioned whether reduced metabolic fitness of autologous CD8⁺ T cells could be observed in transferred CAR T cells. They analyzed CAR⁺CD8⁺ T cells in infusion products of 27 relapsed/refractory CLL patients enrolled in CAR T cell trials. Interestingly, in cases where there was a subsequent complete response, the infused CD8⁺ CAR T cells had increased mitochondrial mass compared with non-responders. This finding is consistent with a previous study that oxidative metabolic features support T cell persistence.[13]

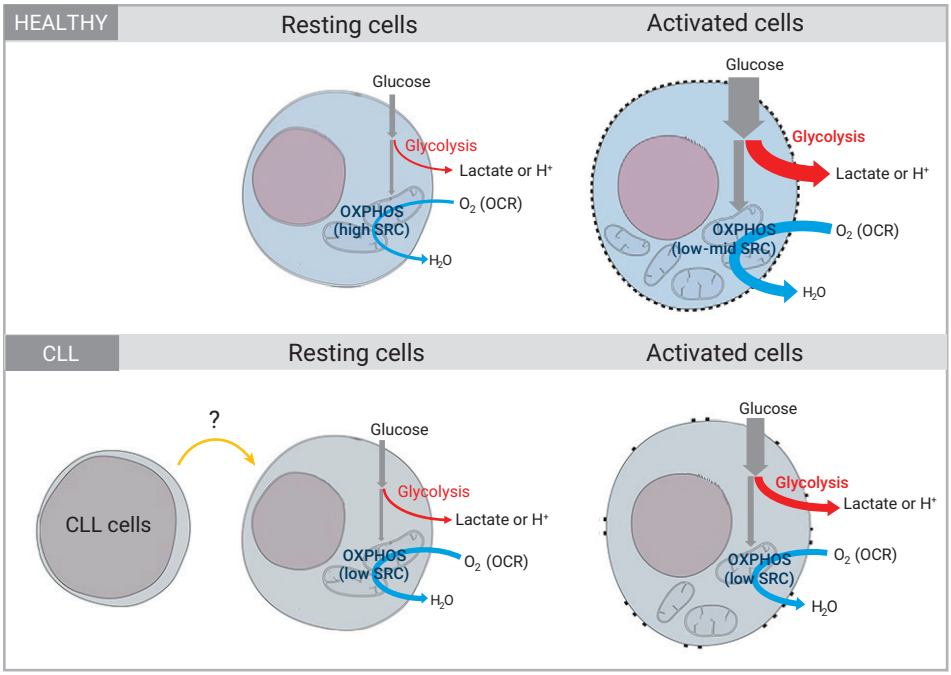

Figure 1. Metabolic strategies in activated T cells. Upon activation, healthy T cells undergo rapid metabolic reprogramming increasing their dependence on glycolysis to meet their increased energetic demands. Studies have shown impairments in glycolysis and oxidative phosphorylation in T cells from chronic lymphocytic leukemia (CLL) patients potentially leading to T cell dysfunction. Figure adapted from van Bruggen *et al.* **2019**.[5]

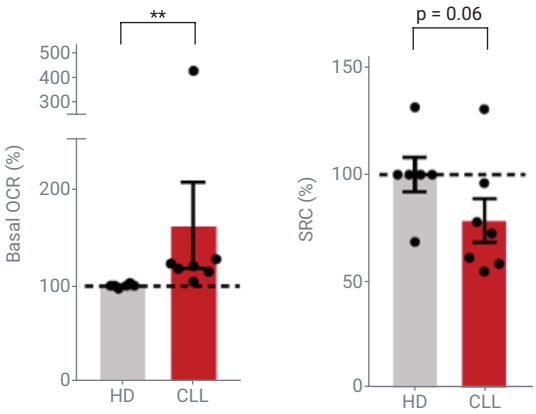

Figure 2. Altered mitochondrial metabolism in CLL-derived CD8⁺ T cells. PBMCs from CLL patients and age-matched HDs were analyzed for OCR (indicating OXPHOS; CLL, n = 7; HD, n = 6) using an Agilent Seahorse XF Analyzer. SRC was calculated as the ratio of maximum OCR over basal OCR (CLL, n = 6; HD, n = 6). Figure adapted from van Bruggen *et al.* **2019**.[5]

These studies led the authors to postulate that impaired mitochondrial health may be central to T cell dysfunction in CLL.[5] The metabolic health of immune cells is therefore a critical determinant that is intrinsically linked to antitumor efficacy. The analysis of immune cell metabolism is of critical importance, both to understanding the biology of the antitumor immune response and for therapeutic design.

Conclusion

This work substantiates a large body of research showing the importance of metabolic processes in immune cell fate, fitness, and function. Agilent Seahorse XF technology, recognized as a leading technology for the study of immune cell metabolism, provides sensitive real-time metabolic measurements. It offers clear insights into the critical drivers behind immune cell function and opportunities to optimize cell therapy products. Agilent now offers a Seahorse XF T Cell Metabolic Profiling kit to assist cell therapy development, providing improved reagents and a streamlined assay workflow. With this kit, a complete picture of T cell energy metabolism can be achieved, providing measurements linked to the antitumor properties of T cell therapy products.

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