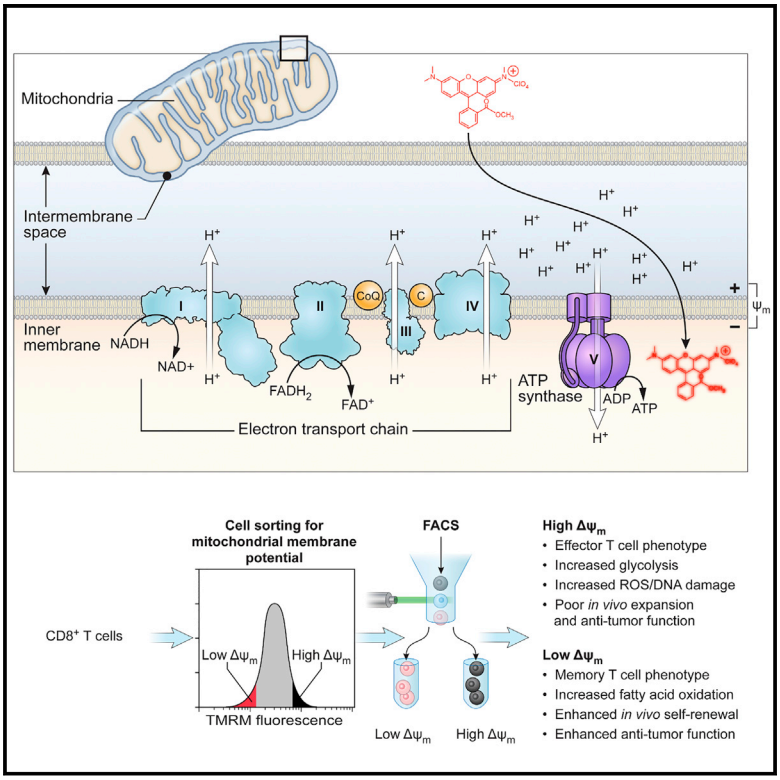
Clinical and Translational Report

Cell Metabolism

Mitochondrial Membrane Potential Identifies Cells with Enhanced Stemness for Cellular Therapy

Graphical Abstract



Highlights

- ΔΨm-based sorting segregates short-lived effector from memory T cell precursors
- Low-ΔΨm $CD8^+$ T cells demonstrate decreased oxidative stress
- Low-ΔΨm T cells demonstrate superior antitumor activity
- Low-ΔΨm marks self-renewing hematopoietic stem cells

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In Brief

Metabolic fitness is required for long-term function of T cells and HSC. Sukumar et al. describe a simple and clinically feasible method to isolate such metabolically robust cells, using a single parameter—mitochondrial membrane potential (ΔΨm)—for long-term survival, antitumor immunity, and hematopoietic reconstitution.

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Mitochondrial Membrane Potential Identifies Cells with Enhanced Stemness for Cellular Therapy

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SUMMARY

Long-term survival and antitumor immunity of adoptively transferred CD8$^+$ T cells is dependent on their metabolic fitness, but approaches to isolate therapeutic T cells based on metabolic features are not well established. Here we utilized a lipophilic cationic dye tetramethylrhodamine methyl ester (TMRM) to identify and isolate metabolically robust T cells based on their mitochondrial membrane potential ($\Delta\Psi$m). Comprehensive metabolomic and gene expression profiling demonstrated global features of improved metabolic fitness in low-$\Delta\Psi$m-sorted CD8$^+$ T cells. Transfer of these low-$\Delta\Psi$m T cells was associated with superior long-term in vivo persistence and an enhanced capacity to eradicate established tumors compared with high-$\Delta\Psi$m cells. Use of $\Delta\Psi$m-based sorting to enrich for cells with superior metabolic features was observed in CD8$^+$, CD4$^+$ T cell subsets, and long-term hematopoietic stem cells. This metabolism-based approach to cell selection may be broadly applicable to therapies involving the transfer of HSC or lymphocytes for the treatment of viral-associated illnesses and cancer.

INTRODUCTION

Immunotherapy using adoptive transfer of tumor-specific T cells mediates durable and complete disease regression in some patients with metastatic cancer (Brentjens et al., 2013; June et al., 2015; Porter et al., 2011; Riddell and Greenberg, 1995). Mounting evidence has shown that metabolism supports and drives many basic features of T cells, including cellular activation, proliferation, differentiation, effector function (Gerriets et al., 2015; Gerriets and Rathmell, 2012; MacIver et al., 2013; Michalek et al., 2011a, 2011b; Pearce et al., 2009, 2013; Sena et al., 2013; Shi et al., 2011), and antitumor immunity. This has led to a growing interest in leveraging this understanding to improve the efficacy of T cell transfer therapies, such as adoptive transfer immunotherapy in the treatment of cancer. In preclincial models it has been shown that highly glycolytic T cells are short-lived after adoptive transfer and have impaired antitumor immunity (Sukumar et al., 2013), whereas T cells with a metabolic profile characterized by elevated fatty acid oxidation (FAO) (Pearce et al., 2009) and enhanced mitochondrial spare respiratory capacity (SRC) have greater long-term survival (van der Windt et al., 2012).

Although there is increasing evidence that metabolism can affect the survival and antitumor function of T cells, identifying a simple and clinically feasible method to isolate T cells with favorable metabolic features has proved challenging. Because mitochondria are the central metabolic organelle in cells, we hypothesized that the measurement of a single mitochondrial-associated parameter may help to identify T cells with a favorable bioenergetic profile that can survive in vivo for long periods after adoptive transfer for T cell-based immunotherapy.

Here, we describe a clinically feasible method to isolate functionally robust T cells based on a single metabolic parameter: mitochondrial membrane potential ($\Delta\Psi$m). Mitochondria produce energy by establishing an electrochemical proton motive force (Δp) across their inner cell membrane, which in turn fuels the synthesis of ATP by driving the proton turbine F0F1 ATPase (Ehrenberg et al., 1988; Sena et al., 2013; Wang and Green, 2012; Weinberg et al., 2015). We show that CD8$^+$ T cells that are found to have low-$\Delta\Psi$m display enhanced in vivo persistence, augmented autoimmunity, and greater antitumor