The essential role of a non-essential amino acid in modulating metabolic fitness to maximize cancer immunotherapies

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Abstract

Understanding the metabolic characters of effector T cells is required to maximize the metabolic fitness and functional robustness of effector T cells in the metabolically-restricted tumor microenvironment (TME). TME represents a dramatic example of metabolic derangement that is hostile to infiltrating immune cells, where the metabolically demanding cancer cells restrict the function of T effector cells by competing for nutrients and by producing immunosuppressive metabolites, resulting in metabolic antagonism. As such, to mediate a robust anti-tumor immune response, T effector cells should adapt to the fluctuations in a wide range of nutrient and amino acids levels imposed by TME. Asparagine (ASN) is one of the most upregulated amino acids following T cell activation and differentiation. ASN is a non-essential amino acid which can be generated by the de novo synthesis through asparagine synthetase (ASNS) or through salvage of extracellular ASN. Here, we envisioned that effector T cells might engage de novo biosynthetic programming to maintain a sufficient level of intracellular ASN to support proliferation in the absence of exogenous ASN, which uses glutamine as a nitrogen donor to convert aspartate into ASN. In addition, ablation of de novo biosynthesis using ASNS-KO, did not affect effector T cell proliferation. However, the combination of these two conditions abolished effector T cell proliferation, confirming that both de novo synthesis or salvaging of extracellular ASN is essential for effector T cell proliferation. Moreover, our metabolomic data revealed that restriction of ASN leads to a reduction of intracellular ASN in WT T cells, but a complete depletion in ASNS-KO T cells. We thus assessed the anti-tumor effect of anti-PDL1 antibody treatment in B16 melanoma-bearing WT and ASNS-KO mice. Our results showed that under metabolically restricted TME, tumor infiltrated ASNS-KO cells couldn’t engage with neither de novo synthesis nor through salvage of extracellular ASN for their survival and effector function, thus inhibiting anti-tumor immunity, whereas WT T effector cells rely on de novo synthesis through ASNS and maintain sufficient levels of ASN in the ASN restricted TME. Together, our data indicate a layer of metabolic plasticity in effector T cells, by engaging de nova ASN synthesis, which is critical for driving T effector cell mediated anti-tumor immunity in the metabolically-restricted TME.

Background

Figure 1: (A) De novo synthesis of Asparagine. (B) Asparagine restriction modulates metabolic reprogramming and effector function of T cells.

Figure 2: The level of amino acids in naive and active murine T cells was determined by GC/MS (A). Murine T cells were activated and cultured in complete or indicated amino acid-deficient medium, proliferation (CFSE), cell surface activation marker (CD86) and viability (7AAD) were determined by FACS (B & C). The level of amino acids in murine T cells in indicated conditions were determined by GC/MS (D).

Figure 3: Marine T cells that were collected at indicated time-points following activation and the level of ASN was determined immunoblot (A). The level of ASN in marine in WT or ASNS-KO T cells was determined by immunoblot and cell proliferation (CFSE) of indicated groups was determined by FACS (B, C). In indicated marine T eff cells, the level of asparagine (Asn), aspartate (Asp) and the central carbon metabolites were determined by GC/MS (C).

Results

Anti-PDL1 therapy in B16 Melanoma

+ anti-PDL1;
1. Tumor growth;
2. Survivals;

Figure 4: The schematic diagram of the anti-PDL1 therapy in syngeneic B16 melanoma model. Tumor growth and Kaplan-Meier survival curves for mice bearing subcutaneous B16 melanoma followed indicated mice model.

Conclusions

- Enabling a minimum intracellular asparagine pool is sufficient for driving T cell proliferation under extracellular asparagine restriction.
- Metabolic plasticity in effector T cells, by engaging de nova ASN synthesis, which is critical for driving T effector cell mediated anti-tumor immunity in the metabolically-restricted TME.

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References
