AGE-ASSOCIATED NAD DECLINE IMPAIRS MITOCHONDRIAL FITNESS AND FUNCTION OF CAR-T CELLS

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Background CAR-T cell therapy has emerged as a key strategy for cancer treatment. The success of CD19-directed CAR-T cells on haematological malignancies has generated a wide interest in the expansion of this approach to solid tumors. However, the clinical outcomes in this context have been unexpectedly poor due to both extrinsic and intrinsic suppressive factors that drive T cell dysfunction within the tumor microenvironment. A fundamental characteristic that contributes to anti-tumor T cell functionality and that determines CAR-T cell efficacy is the maintenance of mitochondrial fitness, which has been shown to sustain memory/stem-like properties and provide prolonged T cell responses. However, mitochondrial dysfunction is a hallmark of aging. How age-associated alterations of immune metabolism affect the outcome of CAR-T cell therapy remains poorly understood.

Methods Here we generate Her2-directed CAR-T cells from young and old mice. We combined in vitro assays with an in vivo Her2-expressing B16 tumour model to investigate the metabolism and functionality of CAR-T cells. Additionally, we validate our findings using human CD19 CAR-T cells from young (<30yo) and old (>70yo) donors.

Results Here, we show that CAR-T cells from old mice display remarkable defects in mitochondrial activity and mass. Furthermore, aged CAR-T cells are unable to reach an appropriate stem-like phenotype, as shown by a lower proportion of CD44+CD62L+TCF1+ CAR-T cells. As a result, aged CAR-T cells are unable to survive in vivo and control tumor growth upon adoptive cell transfer. We unveil that aged CAR-T cells decrease NAD levels which we link to the upregulation of CD38, a cell membrane protein with NADase properties. We further observe that aged CAR-T are not able to respond to NAD boosters and, importantly, that higher CD38 levels are associated with lower mitochondrial activity. Indeed, blockade of CD38 activity using the small molecule 78c restores NAD levels and mitochondrial function when administered in combination with NAD boosters. In vivo, our combination treatment (CD38i + NAD-booster) restores functionality of aged CAR-T cells, promoting long-term CAR-T cell survival and controlling tumor growth as its younger counterparts. Furthermore, we show that also CAR-T cells generated from old human donors display an impaired mitochondrial fitness, which is rescued upon treatment with CD38i + NAD-booster.

Conclusions Our work proposes a model where the age-associated upregulation of CD38 drives NAD decline and mitochondrial dysfunction in CAR-T leading to limited acquisition of memory/stem-like characteristics. We identify CD38 as a promising target to restore stem-like properties of CAR-T cells.

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