Editorial

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Metformin keeps CD8+ T cells active and moving in NASH-HCC immunotherapy

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Liver cancer development is associated with several risk factors, including hepatitis B and C infection, alcohol abuse, exposure to toxins, or metabolic syndrome.[1] While hepatitis B and C infection have shown a stable decrease owing to the success of the hepatitis B vaccine and hepatitis C treatment, the incidence of liver cancer is expected to continue growing until reaching the daunting number of 1 million deaths by 2025, mirroring the stark increase in the obesity pandemic that affects Western countries in particular.[1] In other tumor types, obesity has been paradoxically associated with a better response to immunotherapies[2]; however, a recent study in hepatocellular carcinoma (HCC), the most common form of liver cancer, demonstrated that patients with non-alcoholic steatohepatitis (NASH)-driven HCCs responded poorly.[3] In this issue of *Journal of Hepatology*, Wabitsch *et al.* describe the underlying mechanism behind the impaired response to anti-PD-1 in mice with NASH-HCC and identify a therapeutic strategy that can improve their response to immunotherapies (Fig. 1).

To this aim, the authors used a collection of mouse models of diet-induced NASH-HCC as surrogates for NASH-HCC in patients. Mice were subjected to normal diet, choline-deficient L-amino-defined (CDAA) diet, western diet (WD), or methionine- and choline-deficient (MCD) diet, and then orthotopically transplanted with liver tumor cells or liver metastatic cells. As expected, mice with NASH-HCC did not respond to anti-PD-1 treatment, while mice with HCC in the absence of NASH had a significant tumor reduction, phenocopying the results from the previous study.[3] In order to understand the different sensitivity to anti-PD-1, and due to the prominent role played by CD8+ T lymphocytes in this treatment, the authors analyzed this cell population in the livers of mice with NASH. Initial experiments demonstrated that, as previously reported,[3] mice with NASH had a high hepatic infiltration of effector CD8+ T cells. Unexpectedly, when analyzing CD8+ T-cell infiltration in the tumor, similar levels were observed in mice with or without NASH. Going beyond cell numbers, the authors studied function-related phenotypic changes, but then again, the proportions of effector CD8+ T cells expressing PD-1, IFN-gamma, and GrmzB remained unchanged in tumors of NASH mice, excluding this type of alteration as an explanation for the poor response observed in these animals.

Given that the conventional parameters associated with CD8+ T-cell function, such as CD8+ T-cell numbers or functionality, were not altered, the authors explored the tumor microenvironmental dynamics *in vivo* by performing intra-vital imaging. "Seeing is believing" becomes truer when the sophisticated live observation of cells *in vivo* is accompanied by detailed functional characterizations. This underutilized approach revealed that intratumor CD8+ T cells in mice with NASH had a lower speed and a shorter displacement length compared to intratumor CD8+ T cells in mice without NASH. An opposite phenotype of increased motility was observed in CXCR6+CD8+ T cells, which is a population of CD8+ T cells known to promote NASH[4]; how NASH affects different CD8+ T-cell populations, their motility, and activity remains an unanswered question. Of note, the reduced motility of CD8+ T cells was not restricted to liver tumors as it was also observed in intrahepatic T cells as well as in cells infiltrating tumors growing in a different anatomical location (subcutaneous). However, the impairment in lymphocyte motility was more marked in lymphocytes infiltrating hepatic tumors, highlighting the specificity of this phenotype. Accordingly, the efficacy of anti-PD-1 immunotherapy was not impaired in the case of subcutaneous tumors in mice with NASH, indicating a more severe impairment in the liver microenvironment and an association between efficacy of anti-PD-1 immunotherapy and T-cell motility in the liver.

Cell motility and trafficking is usually governed by chemokines and adhesion molecules, which dictate T-cell homing. Moreover, immune checkpoint molecules, including PD-1, have been shown to modulate T-cell motility.[5] However, in addition to

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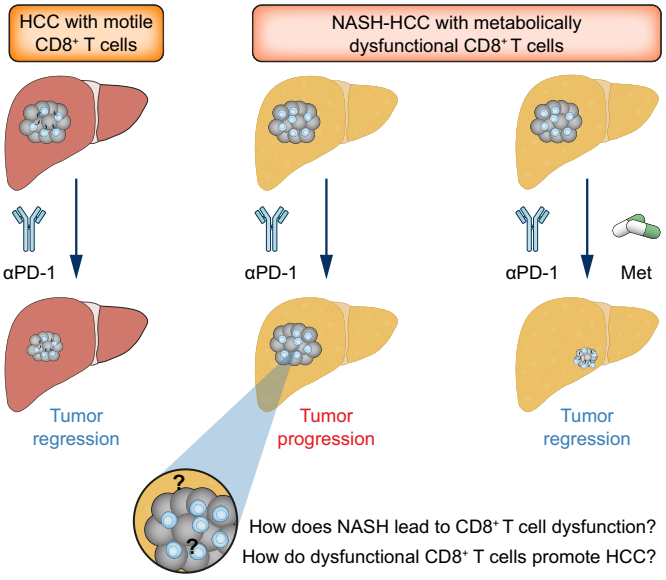

Fig. 1. Schematic depicting the metabolic dysfunction of CD8+ T cells in NASH-HCC and its reversal by metformin. Grey, tumor; blue, CD8+ T cells. HCC, hepatocellular carcinoma; Met, metformin; NASH, non-alcoholic steatohepatitis. (This figure appears in color on the web.)

similar levels of PD-1, in the case of CD8+ T cells in NASH mice, the reduced motility was independent of chemotaxis or cell adhesion, suggesting a cell-intrinsic mechanism. To better understand the impaired motility of CD8+ T cells, and considering the importance of metabolism in cell motility, an exhaustive transcriptomic characterization focused on metabolic genes was performed. CD8+ T cells from NASH-HCC livers presented alterations in several metabolic pathways, including glycolysis, fatty acid oxidation, and mitochondrial respiration, which at the same time were associated with mitochondrial depolarization and loss of mitochondrial mass. Specifically, 2-NBDG uptake was significantly reduced in hepatic CD8+ T cells in mice with NASH-HCC that were treated with anti-PD-1.

Since metformin can help regulate glucose metabolism and had previously been shown to reprogram CD8+ T cells,[6] the authors tested the effect of metformin on the activation and motility of CD8+ T cells. While metformin did not affect mitochondrial polarization, the drug led to a significant increase in mitochondrial mass and activation. Most importantly, metformin treatment restored the motility and speed of CD8+ T cells in NASH-HCC livers. Finally, metformin was able to restore the sensitivity of NASH-HCC tumors to immunotherapies. Metformin synergized with anti-PD-1 monotherapy or with combination immunotherapy of anti-VEGFR2 and anti-PD-L1, which emulates the standard-of-care for patients with HCC, demonstrating the translational potential of this “old” drug. The effect of metformin was mainly associated with the increased activity to CD8+ T cells rather than a reduction in NASH, as only small changes were observed in steatosis, serum glucose, or ALT levels.

Tumors generated in NASH mice display many features of those found in patients with NASH. However, orthotopic implantation of tumor cells may not fully recapitulate the immune response that is elicited in autochthonous tumors developed in patients. Despite this limitation, the current study has important translational implications. First of all, metformin is a drug with limited toxicity that has been used for decades in the treatment of type 2 diabetes, so it could easily be utilized in patients with NASH-HCC. Second, the study provides a mechanistic insight into the impaired response of patients with NASH-HCC to immunotherapy, where T-cell motility and their related metabolic parameters, but not infiltration, are associated with anti-PD-1 efficacy. Additional work will be required to directly demonstrate that the restored antitumor capacity of highly motile CD8+ T cells results from metformin treatment. Nevertheless, the lack of differences in immune-related functions in NASH CD8+ T cells suggests that their restored motility may be behind the enhanced therapeutic efficacy of anti-PD-1. Interestingly, clinical data demonstrate that T-cell infiltration is the main feature that determines sensitivity to PD-1 blockade and studies in patients with HCC have suggested that increased CD3 and CD8 levels and inflammatory gene signatures are associated with improved overall survival after nivolumab treatment.[7] The lack of data regarding the proportion of patients with NASH-HCC in the cohort that was used for these clinical studies precludes us from reaching conclusions about tumor inflammation and response to PD-1 blockade in these individuals.

Additional studies will be needed to validate whether or not patients with NASH-HCC harbor CD8+ T cells and whether or not these CD8+ T cells present metabolic defects. The metabolic characterization of CD8+ T cells beyond their mere presence may be needed to better stratify patients and to select the most effective therapies. Another interesting question is whether metformin could be used in other tumor types to improve response to anti-PD-1 therapy. In the current study, the effects of metformin seem to be specific to CD8+ T cells in the liver, suggesting the unique biology of these cells in the liver, an organ that is considered immune-privileged. Moreover, in addition to CD8+ T lymphocytes, there are other immune and non-immune cell subsets (*e.g.* dendritic cells, macrophages) that are not the main effectors in PD-1-blocking therapies but are involved in their efficacy [8]: the role of these cells in resistance to these therapies in NASH and the potential effect of metformin are currently unknown and remain to be addressed. However, additional studies in other tumor types and even in other etiologies in the context of liver cancer may broaden the therapeutic applicability of metformin.

Finally, why CD8+ T cells undergo metabolic reprogramming in the context of NASH-HCC is still unknown. The direct effects of obesity-inducing diet combined with the altered interactions between tumor cells and cells in the microenvironment may be at play. In addition, changes in the microbiome may also affect CD8+ T-cell biology, as the microbiome of patients with NASH-HCC is significantly different from that in patients with non-NASH-HCC.[9,10] It was previously demonstrated by the same team that NASH can affect CD4+ T cells[11] and CD4+ T cell-dependent immunotherapy in HCC.[12] This new study goes one step further and identifies another detrimental side effect of NASH in liver cancer biology. Future studies will help confirm the potential of metformin for NASH-HCC immunotherapy and its translatability into other tumor types.

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Conflict of interest

AL has received research funding from Pfizer and Genentech and consulting fees from Astra Zeneca. PS declares no conflict of interest.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Both authors contributed equally.

Supplementary data

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