Article

NASH limits anti-tumour surveillance in immunotherapy-treated HCC

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Hepatocellular carcinoma (HCC) can have viral or non-viral causes[1–5]. Non-alcoholic steatohepatitis (NASH) is an important driver of HCC. Immunotherapy has been approved for treating HCC, but biomarker-based stratification of patients for optimal response to therapy is an unmet need[6,7]. Here we report the progressive accumulation of exhausted, unconventionally activated $CD8^+PD1^+$ T cells in NASH-affected livers. In preclinical models of NASH-induced HCC, therapeutic immunotherapy targeted at programmed death-1 (PD1) expanded activated $CD8^+PD1^+$ T cells within tumours but did not lead to tumour regression, which indicates that tumour immune surveillance was impaired. When given prophylactically, anti-PD1 treatment led to an increase in the incidence of NASH–HCC and in the number and size of tumour nodules, which correlated with increased hepatic $CD8^+PD1^+CXCR6^+$, TOX^+, and TNF^+ T cells. The increase in HCC triggered by anti-PD1 treatment was prevented by depletion of $CD8^+$ T cells or TNF neutralization, suggesting that $CD8^+$ T cells help to induce NASH–HCC, rather than invigorating or executing immune surveillance. We found similar phenotypic and functional profiles in hepatic $CD8^+PD1^+$ T cells from humans with NAFLD or NASH. A meta-analysis of three randomized phase III clinical trials that tested inhibitors of PDL1 (programmed death-ligand 1) or PD1 in more than 1,600 patients with advanced HCC revealed that immune therapy did not improve survival in patients with non-viral HCC. In two additional cohorts, patients with NASH-driven HCC who received anti-PD1 or anti-PDL1 treatment showed reduced overall survival compared to patients with other aetiologies. Collectively, these data show that non-viral HCC, and particularly NASH–HCC, might be less responsive to immunotherapy, probably owing to NASH-related aberrant T cell activation causing tissue damage that leads to impaired immune surveillance. Our data provide a rationale for stratification of patients with HCC according to underlying aetiology in studies of immunotherapy as a primary or adjuvant treatment.

Potentially curative treatments for HCC, such as liver transplantation, tumour resection, or ablation, are limited to early-stage tumours[1,2]. Multikinase inhibitors and anti-VEGF-R2 antibodies have been approved for use in advanced HCC[1,2]. Immunotherapy, which is thought to activate T cells or reinvigorate immune surveillance against cancer, showed response rates of 15–30% in patients with HCC[5,8–11]. Nivolumab and pembrolizumab (PD1-directed antibodies) have been approved for treatment of HCC[3,4], although phase III trials failed to reach their primary endpoints to increase survival[1,10,11]. A combination of atezolizumab (anti-PDL1) and bevacizumab (anti-VEGF) demonstrated increased overall and progression-free survival in a phase III trial, making it a first-line treatment for advanced HCC[5]. The efficacy of immunotherapy might be affected by different underlying HCC aetiologies, with diverse hepatic environments distinctly regulating HCC induction and immune responses[6]. Hence, we lack biomarkers that correlate with treatment response to allow patient stratification[12,13]. Non-alcoholic fatty liver disease (NAFLD) is an HCC-causing condition that affects more than 200 million people worldwide[14]. Approximately 10–20% of individuals with NAFLD progress over time from steatosis to NASH[14]. Innate and adaptive immune-cell activation[15–17], in combination with increased metabolites and endoplasmic reticulum stress[16,18], are believed to lead to a cycle of hepatic necro-inflammation and regeneration that potentially leads to HCC[19–21]. NASH has become an emerging risk factor for HCC[1,14,19], which led us to investigate the effects of immunotherapy in NASH–HCC[22–24].

Hepatic $CD8^+PD1^+$ T cells increase in NASH

We fed mice with diets that cause progressive liver damage and NASH over 3–12 months (Extended Data Fig. 1a–c), accompanied by an increase in the frequency of activated $CD8^+$ T cells expressing CD69, CD44 and PD1 (Extended Data Fig. 1d–g). Single-cell mapping of leukocytes showed altered immune-cell compositions in mice with NASH (Extended Data Fig. 1h, i) with strongly increased numbers of $CD8^+PD1^+$ cells (Fig. 1a, b, Extended Data Fig. 1j–m, o). Similarly, elevated $CD8^+$ and $PD1^+$ cells were found in a genetic mouse model of NASH[17] (Extended Data Fig. 1n). Messenger RNA in situ hybridization and immunohistochemistry showed that increasing PDL1 expression in hepatocytes and

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