DISTINCT MACROPHAGE METABOLISM AND PHENOTYPIC CHANGES IN VIRAL AND NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD) RELATED HEPATOCELLULAR CARCINOMA

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Background Different aetiologies of hepatocellular carcinoma (HCC) may influence the tumour microenvironment with implications for immunotherapy. Macrophages, especially the resident Kupffer cells (KCs), are the major PD-L1+ liver cells, and their pro- or anti-inflammatory functions directly affect the progression from chronic inflammation to malignancy. Previous studies have shown that KCs in HCC are associated with poorer survival. We hypothesise that KCs in HCC possess altered lipid metabolism and immunosuppressive phenotype, potentially leading to distinct immunotherapy responses in NAFLD-HCC patients.

Methods Using light sheets microscopy and flow cytometry, we characterise lipid accumulation and macrophage phenotypes in both the tumour and the adjacent non-tumour for 5 viral and 5 non-viral HCC patients. We then validate the association between lipid accumulation and macrophage phenotype in vitro with KCs differentiated from induced pluripotent stem cells (iPSCs) or peripheral blood mononuclear cells (PBMCs). iPSC and patient-derived HCC organoids are then obtained and are co-cultured with KCs and T cells (if needed), which are further characterised with confocal microscopy, multiplex cytokine assay (Luminex) and single cell RNA sequencing (scRNA-seq).

Results Light sheets microscopy of patient samples shows co-localisation between areas of high lipid accumulation and high PD-L1 expression. In the macrophages, flow cytometry confirms a positive association between high lipid accumulation and M2-like phenotype, which is more highlighted in the non-viral HCC patients. In vitro experiments show lipid accumulation in KCs caused by HCC tumour cells, directly contributing to an immunosuppressive phenotype. Strikingly, we also observe increased lipid accumulation in tumour cells and patient-derived tumour organoids, which is KC-specific. Such cycle of lipid accumulation and immunosuppressive changes is possibly mediated by the presence of both pro- and anti-inflammatory cytokines in the culture, as shown by Luminex. This could lead to increased de novo fatty acid synthesis in the KCs following tumour cell/organoid co-culture, as highlighted in scRNA-seq. We have also established an organoid-KC-T cell co-culture model to characterise T cell dysfunction.

Conclusions These results show that lipid accumulation in the HCC macrophages are associated with an immunosuppressive phenotype, which could depend on the disease aetiology. We have constructed an HCC organoid model that recapitulates the patient macrophage phenotypes, providing a platform for downstream mechanistic studies. Co-culture of tumour organoids and KCs lead to lipid accumulation in both cell types, which in turn may contribute to T cell dysfunction. Such effect may be due to the de novo lipogenesis downstream of the cytokine milieu.

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Ethics Approval Patient samples were obtained with ethics approval under the grant NMRC/OFLCG/003/2018, according to the guidelines of the SingHealth Central Institutional Review Board. Participants have given informed consent before taking part.

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