SAT-557-YI

Loss of fibronectin contribute to poor maturation of monocyte to resolving macrophages in acute and chronic liver injury

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Background and aims: Loss of liver macrophage number and their function have been shown to be associated with poor liver clearance in both acute and chronic liver injury; however, the underlying mechanism is not well defined. The extracellular matrix is a dynamic structural component of tissue and regulate cell behaviour during tissue repair and regeneration. In the current study, we aim to study the role of the liver matrix in the maturation of bone marrow derived monocytes into macrophages and their phenotypic polarisation as well as function.
Method: Human healthy (n = 4), acute liver failure (ALF; n = 5), and chronic liver disease (CLD; n = 5) decellularized liver Extracellular matrix (ECM) was used for label-free quantitative matrix proteome using LC-MS. The decellularized matrices were lyophilized and solubilized for in vitro culture of macrophages. Gelatin and human plasma fibronectin were dissolved and coated over the tissue culture plates (TCP) for monocyte-macrophage culture. Cellular parameters like maturation, phenotype, cytokine release, and functionality were studied by flow cytometry.
Results: To check whether liver ECM has any role in maturation, polarization, and functionality of macrophages, we cultured human peripheral blood monocytes over healthy, ALF, and CLD human liver ECM solution-coated and uncoated TCPs and found that cells cultured over healthy ECM has significant high expression of maturation marker (25f9; $p < 0.001$), resolving macrophage markers ($p < 0.05$; CD163, CD206), and anti-inflammatory cytokine IL-10 ($p < 0.001$), and lower expression of inflammatory cytokines TNF-alpha ($p < 0.01$) and IL-1beta ($p < 0.01$). To further identify the factors that are regulating macrophages, we did a proteomic analysis of decellularized healthy, ALF and CLD human liver ECMs and found that out of 277 shared proteins, fibronectin was downregulated in the ALF and CLD matrix proteomes. For validating the effect of fibronectin on macrophage regulation, we coated the TCP with fibronectin and cultured monocyte-macrophages over it. In-vitro culture of human macrophages over fibronectin showed significantly higher expression of 25f9 ($p < 0.001$), resolving macrophage markers CD163 ($p < 0.05$), CD206 ($p < 0.01$), and a significant increase in their phagocytosis ($p < 0.01$) and efferocytosis ($p < 0.001$) activity as compared to gelatin coated and TCP. Also, the expression of pro-inflammatory cytokines (TNF-alpha and IL-1beta, $p < 0.01$) was significantly lesser in the fibronectin group, while the expression of anti-inflammatory cytokines (IL-10) was significantly higher ($p < 0.001$) when cultured on fibronectin coated plates.
Conclusion: The loss of fibronectin in ALF and CLD may contribute to the loss of macrophage maturation and function. Providing fibronectin enriched matrix may serve as tool for macrophages polarisation for tissue engineering.

SAT-558

CD8+ effector T cells expressing low levels of CD127 upregulate GPR56 expression and reside in the hepatic autoimmune and inflammatory microenvironment

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Background and aims: In contrast to liver cancer and viral hepatitis, little is known about the pathogenic or dysfunctional effector T cells within the proinflammatory microenvironment in inflammatory and autoimmune liver diseases.
Method: Bulk RNA-sequencing and flow cytometric analyses allowed us to identify a specific subset of $CD8^+$ T cells in both blood and liver. Immunohistochemistry analysis was performed to detect cell localisation and ligand identification.
Results: We observed a $CD8^+$ T cell subset which displays a low level of CD127 and CD160 expression. This population concomitantly upregulates the immune checkpoint GPR56 (25.76% ±4.21), an adhesion G-protein-coupled receptor, whose ligand, type 3 collagen, is particularly abundant in fibrotic and cirrhotic livers. We performed multicolour flow cytometry analyses on liver-derived lymphocyte T cell populations isolated from end-stage diseased livers with different aetiologies (ALD, n = 2; MASH, n = 4; AIH, n = 1; PSC, n = 2) as compared to non-cirrhotic donor livers not suitable for transplantation (controls, n = 2). Another hallmark of this $CD127^{lo}$ $CD8^+$ T cell population is their low expression of the transcription factor TCF-1, consistent with their acquisition of effector properties, potentially sustaining persistent inflammatory processes. $GPR56^+$ $CD127^{lo}$ $CD8^+$ T cell recruitment and concentration around the portal tract regions could be facilitated by the expression of CXCR3 and CCR6. Immunohistochemistry analysis suggested that these cells reside around fibrous septa expressing collagen 3 and may create a proinflammatory niche establishing a chronic inflammatory disorder. *Ex vivo* assays with flow cytometry staining will help characterise their proinflammatory cytokine ($TNF\alpha$, $IFN\gamma$) and effector molecule (granzyme, perforin) secretion profile, alongside spatial transcriptomic analysis which will enable a deeper description of this newly discovered T cell subset.
Conclusion: We identified a new subset of $CD127^{lo}$ $CD8^+$ T cells with a pro-inflammatory cytokine profile and residency to fibrous stromal framework. These T cells may potentially be involved in the pathogenesis of autoimmune and inflammatory liver diseases.

Liver transplantation and hepatobiliary surgery – Basic

WED-008

Adipose tissue as a predictor of morbidity and mortality in patients listed for liver transplantation

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Background and aims: Body composition plays a crucial role in the prognosis of liver diseases. However, recent studies have focused mainly on muscle parameters and less is known about the adipose tissue (AT) (=subcutaneous AT (SAT) and visceral AT (VAT)) in terms of prognosis. The aims of our work are to determine the impact of body composition on the morbidity/mortality of patients listed for liver transplantation (LT) and to assess the evolution of these parameters 6 months post-LT.
Method: This single-center prospective observational study screened adult patients who were assessed for LT from June 2021 to October 2023. Clinical data including nutritional and functional status, radiological assessment of muscular compartment and AT were performed at baseline and 6 months post-LT. Measures were

performed on abdominal CT scan at the third lumbar level (L3) using the Slice-O-Matic software. The composite outcome was defined as death and/or emergency admission to hospital. Uni- and multivariate Cox proportional hazard regressions were computed to identify predictors of morbidity and mortality. T-test investigated the evolution of variables between pre- and 6-months post-LT. Spearman correlations were also performed.

Results: We included 158 patients with a mean MELD score of 16 and a mean age of 54 years. 74% were males. 124 were cirrhotic, with alcohol-related cirrhosis in 49.2 and metabolic-associated cirrhosis in 11.3%. 35.2% were listed for hepatocellular carcinoma. During the study period, 58.2% of the patients were transplanted, 63% of them being assessed at 6-month post-LT. During the follow-up, 9, 5% died while waiting for LT, all being cirrhotic. There was at least one acute admission for 28.5%. In univariate analysis, the following variables were significantly associated with the composite outcome : MELD (HR 1.15; IC 1.1–1.201; $p < 0.005$), liver frailty index (LFI) (HR 2.73; IC 1.688–4.408; $p < 0.005$)), density of SAT (HR 1.05; IC 1.028–1.072; $p < 0.05$)), density of VAT (VAT; HR 1.04; 1.011–1.072, $p = 0.007$) and muscle density (HR 0.97; 0.956–0.987; $p < 0.005$). In multivariate analysis, only MELD and LFI were associated with outcome (HR: 1.14; IC1.078–1.20; $p < 0$, 005 and HR: 2.054; IC 1.176–3.588; $p = 0.01$; respectively). Among body composition characteristics, density of SAT was the strongest predictive factor but didn't reach significance (HR 1.033, $p = 0.091$). There was a strong correlation between density of AT and the MELD score (SAT: $r = 0.598$, 95% CI 0, 4747–0, 6987, $p < 0.0001$; VAS: $r = 0.5615$, 95% CI 0, 4306–0, 6692, $p < 0.0001$). At the 6-month post-LT, density of SAT decreased significantly ($p < 0.005$), while density of muscle increased ($p = 0.019$).

Conclusion: High SAT density, compatible with tissue inflammation, is associated with complications on the LT list and improves after LT. However, the MELD score and LFI remain the most robust independent factors for morbidity/mortality.

WED-009

Cytomegalovirus specific polyfunctional t-cells control CMV reactivation, after liver transplantation

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Background and aims: Cytomegalovirus (CMV) viral load after liver transplantation (LT) is controlled by cell mediated immune responses (CMI). Quantification of CMV-specific T-cells may allow identifying patients that achieve spontaneous CMV control. In addition, CMI could be used to measure the state of immune-competence status of the patient, and thus useful as a biomarker to predict the risk of infections/reactivations, rejection and *de novo* tumors.

Method: Prospective post-LT clinical, virologic and immunological monitoring was carried out up to 1 year in a cohort of adult LT recipients (LT-R). CMV-specific responses were characterized using flow-cytometry multiparametric analyses.

Results: We analyzed 518 samples from 104 LT-R (83.65% R+, 16.35% R−). CMV reactivation occurred in 52 patients, 36 of which (69.23%) spontaneously controlled viral replication, while 33 had severe reactivation, 78.79% of which was developed in patients at low risk of CMV reactivation (7 in R− and 26 in R+). In pre-LT samples, 48.1% and 56.7% of the patients had detectable CMV-pp65 CD8+ or CD4+ T-cell response, respectively (at least one cytokine). In contrast, only 24.0% or 16.3% had detectable CMV-specific CD8+ or CD4+ polyfunctional T-cells, respectively. Patients with CMV-specific CD4+ T-cells higher than 0.05% of total T-cells pre-LT had less CMV reactivation episodes ($p = 0.0018$), and less severe CMV events ($p = 0.0043$). Only patients with undetectable CMV-specific CMI pre-LT developed CMV infection and were diagnosed with severe CMV events. No statistically significant differences were detected between polyfunctional CMI driven by CD4+ and CD8+ T-cells in patients who developed a de novo tumor, an acute cellular rejection or other infections and those who did not.

Conclusion: While the quantitation of CMV-specific CMI, especially that driven by CD4+ T-cells, showed good diagnostic performance to predict CMV reactivation, it did not predict the development of other clinical events (other infections, *de novo* tumors and rejection). CMV-specific CMI may be a useful biomarker for spontaneous control of CMV reactivation to tailor antiviral treatment after LT.

WED-010

Functions and molecular mechanisms of metformin in ameliorating ischemia-reperfusion injury of steatosis liver by inhibiting ferroptosis

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Background and aims: Liver transplantation is the only effective treatment for end-stage liver disease. However, the shortage of donors seriously restricts the development of liver transplantation. In recent years, there has been an increasing proportion of steatosis liver in the donor pool. Compared to normal livers, steatosis livers are more susceptible to ischemia-reperfusion injury (IRI), which greatly affects the outcome of liver transplantation. Nevertheless, the underlying mechanisms and corresponding improvement strategies remain largely unexplored. Previous studies have demonstrated that metformin, a first-line drug for type 2 diabetes management, not only effectively reduces blood sugar levels but also significantly improves stroke, myocardial infarction, and renal IRI. However, limited research has been conducted on metformin's role in liver IRI. Therefore, my study aims to investigate whether metformin can mitigate IRI in steatosis livers and elucidate its associated molecular mechanisms.

Method: We utilized clinical samples collected before and after liver transplantation from donors as well as non-alcoholic fatty liver mouse models with hepatic ischemia-reperfusion injury (HIRI) and cell models of non-alcoholic fatty liver to evaluate the effects of metformin in mitigating IRI via inhibiting ferroptosis. Mass spectrometry analysis was employed alongside Chromatin Immunoprecipitation (ChIP) assays and adeno-associated virus (AAV-8) precipitation techniques to identify and validate the potential regulatory mechanisms.

Results: Steatosis liver is indeed more prone to HIRI and exhibits a higher degree of ferroptosis. Treatment with metformin can significantly alleviate the IRI of steatosis liver. Mass spectrometry showed that metformin can down-regulate the level of transcriptional coactivator P300. Corresponding mechanism studies showed that metformin promoted the degradation of P300 by activating AMPK. Further ChIP-seq results showed that P300 could bind to the promoter region of ACSL4, up-regulate its H3K27ac modification, and promote the expression of ACSL4. Overexpression of P300 or ACSL4 can significantly reverse the improvement of metformin on the steatosis liver IRI.

Conclusion: This study reveals that metformin can effectively alleviate steatosis liver IRI through AMPK/P300/ACSL4 pathway, providing an important theoretical basis for clinical use of metformin to reduce the occurrence of steatosis liver IRI and improve its utilization rate in liver transplantation.