A patient-derived model to study how the extracellular matrix modulates anti-tumour immunity in hepatocellular carcinoma

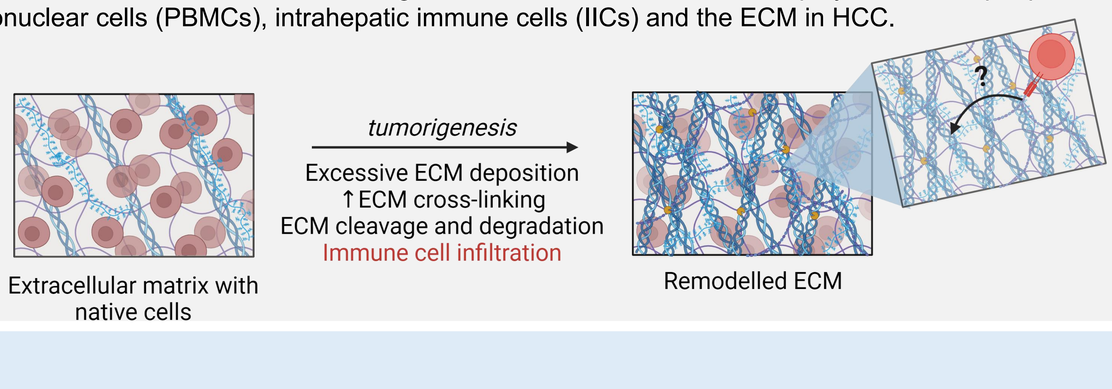
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Background

Immune-targeted therapies, specifically immune-cell-directed approaches, are becoming central to treatment strategies for patients with hepatocellular carcinoma (HCC). Produced primarily by cancer-associated fibroblasts, the extracellular matrix (ECM) is a dynamic regulator of the immune system. In cancer, it is actively remodelled and dysregulated, influencing immune cell infiltration, cytokine signalling and function. Recent studies increasingly demonstrate that ECM proteins exhibit immunomodulatory functions within the liver microenvironment, yet their specific role in modulating immune phenotypes present in HCC remains unclear.

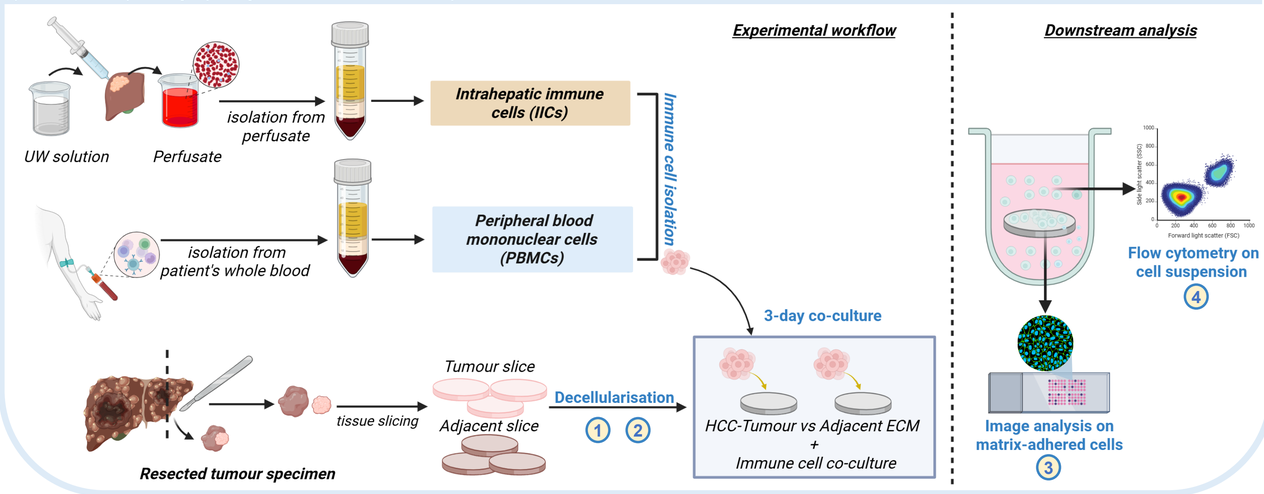
Here, we explore the direct impact of ECM proteins on T-cell responses in patient-derived models of HCC to understand their role in immune regulation. We examine the interplay between peripheral blood mononuclear cells (PBMCs), intrahepatic immune cells (IICs) and the ECM in HCC.



Methods

Exploring the immunomodulatory effects of tumour ECM on HCC-immune cells

To study the remodelled tumour ECM, we developed a 3D co-culture system using decellularised tumour and adjacent liver precision-cut slices (PCTS/PCLS) and lymphocytes derived from HCC patients.



Results

1 Cellular content was effectively eliminated from tissues through decellularisation, maintaining the ECM composition and structure

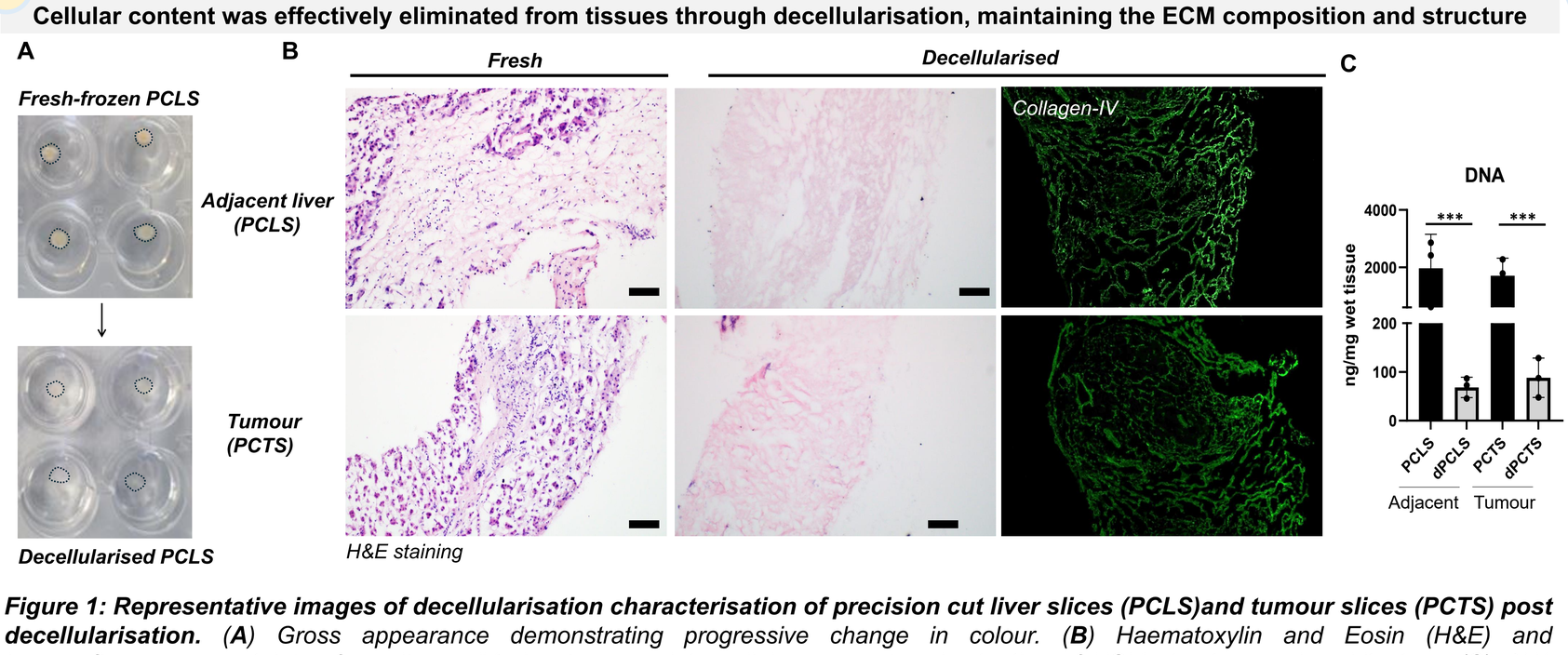


Figure 1: Representative images of decellularisation characterisation of precision cut liver slices (PCLS)and tumour slices (PCTS) post decellularisation. *(**A**) Gross appearance demonstrating progressive change in colour. (**B**) Haematoxylin and Eosin (H&E) and immunofluorescence staining for collagen IV showing complete cellular removal and retention of ECM structure and architecture. (**C**) DNA quantification showing sufficient reduction in DNA content.*

2 Proteomics reveals differences in core and associated ECM proteins between liver adjacent and HCC decellularised tissues

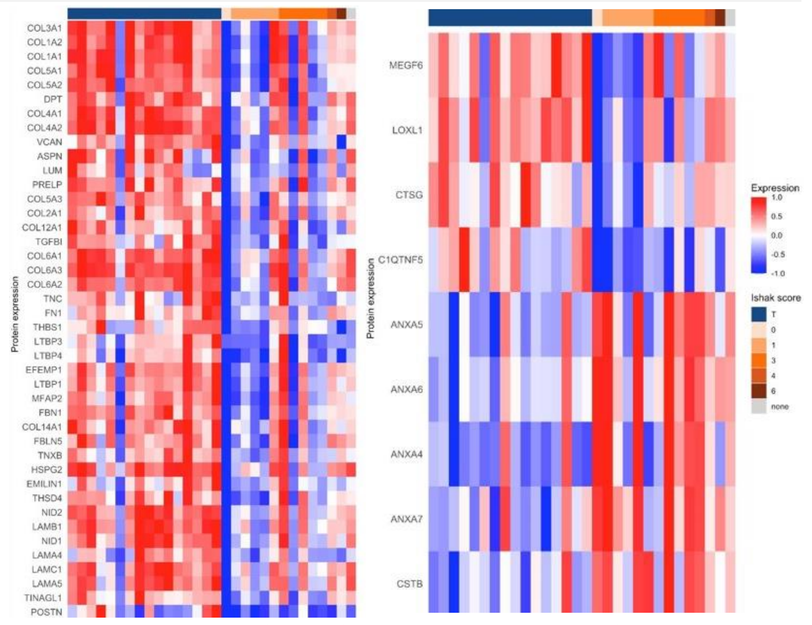


Figure 2. Heatmap of significantly upregulated ECM proteins *in adjacent (n=15) and HCC tumour (n=13) tissues showing differences in core (left) and ECM-associated (right) genes between the tissue.*

3 After 3-day co-cultures, distinct localisation patterns of immune cells were observed between tumour and adjacent ECM, revealing ECM-driven immune cell and T cell interactions

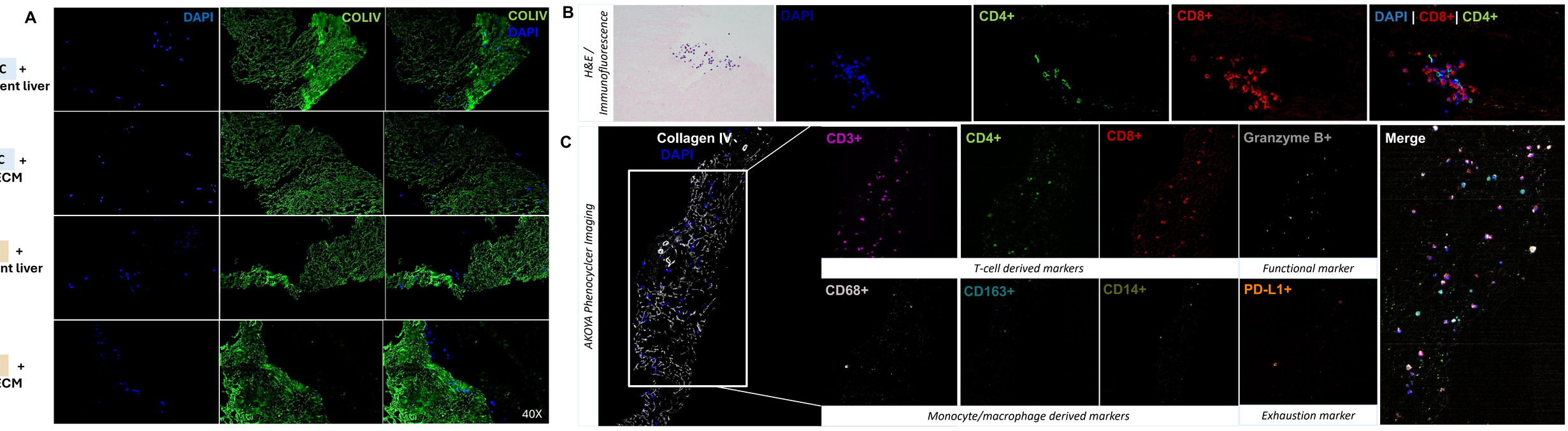


Figure 3. *(**A**) DAPI and Collagen IV immunofluorescence staining illustrating the distribution of PBMCs and IIC on the ECM extracts, after 3 days of culture.(**B**) PBMCs co-cultured with adjacent-liver ECM. H&E staining immunofluorescence staining for CD4 andCD8 demonstrates the presence of T-cell populations (n=1). (**C**) High-dimensional spatial immune profiling of PBMC's co-cultured with adjacent-liver ECM.*

4 Immunophenotyping HCC-immune cells upon exposure to ECM-derived HCC and adjacent ECM tissue

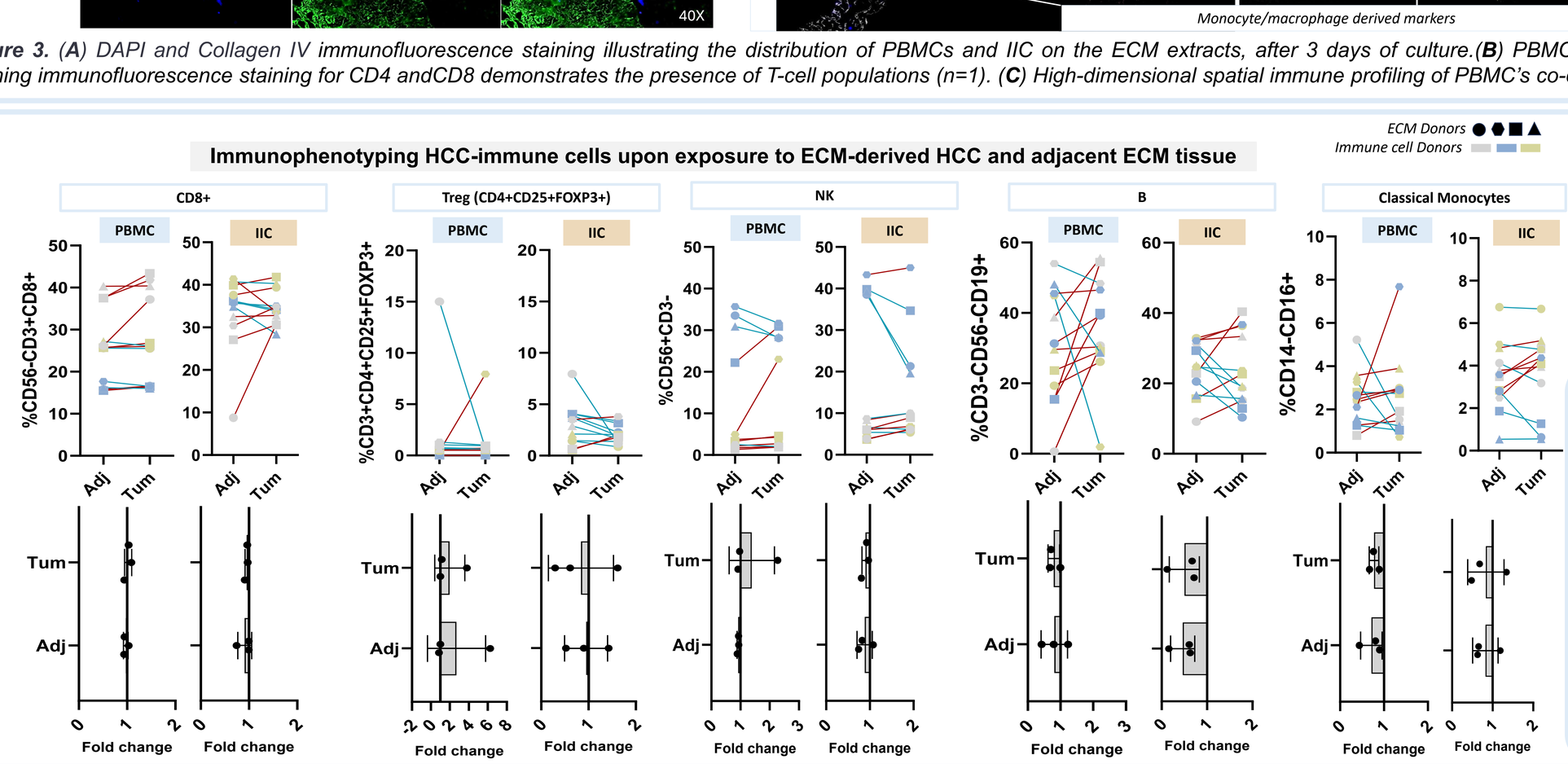


Figure 4. *Flow cytometry plots of immune cell subsets of PBMCs and IICs upon exposure to n=4 ECM donors (top), red lines indicate increase and blue lines indicate decrease. Comparing differences amongst immune cell donors against adjacent and tumour ECM, normalised to media control (bottom), Data presented as mean values +/− standard deviation (SD).*

Conclusion

- We developed a patient-derived model to study how immune cells interact with the ECM in HCC, revealing altered immune cell distribution in response to ECM exposure.
- Proteomic analysis shows distinct differences in matrix protein composition between HCC and adjacent tissue.
- T cells and macrophages interact directly with the ECM, suggesting a role for dysregulated ECM in shaping immune behaviour.