ANALYSIS OF TUMOR-IMMUNE ASSEMBLOIDS FOR CANCER IMMUNOTHERAPY

Authors

Echo Yongqi Luo, Laidlaw Scholar, The University of Hong Kong
Major in Molecular Biology and Biotechnology and Neuroscience
Member of the Young Scientist Scheme, The Faculty of Science

Affiliations

Sincere gratitude to Dr Rio Sugimura for his instructive supervision and Miss Ritika Priyul Jogani for her attentive demonstrations during the whole research progress in the BEL lab. This research project is part of the Laidlaw Scholar Programme 2021-2022.

Hepatocellular carcinoma (HCC) is the third deadliest cancer globally. HepG2-derived tumours, recapitulating the cancerous cell sorting and spatial lineage restriction in vivo, are ideal 3D in vitro models for characterisation and anti-cancer therapy screening of HCC. However, HepG2 tumours alone are limited by the lack of vasculature and tissue-resident immune cells, which play indispensable roles in cancer progression. To recapitulate HCC's microenvironment, we incorporated human HepG2-derived tumours with human EPSC-derived vascular immune organoids (VIO) to form assembloids that fully encapsulate the physiological characteristics of hepatic cancers. Fluorescent analyses demonstrated a satisfying fusion between HepG2 tumours and VIOs, with heterogeneous morphological features. Overall, our study generated an ideal HCC assembloid model and provided deeper insights into the tumour-immune interactions in HCC, thereby facilitating the improvement of HCC-targeting immunotherapies (e.g. CAR-T, anti-CTLA/PD-L1).

INTRODUCTION

HepG2 is the most popular hepatic cell line for liver oncogenesis research. Derived from human hepatoblastoma and characteristically mutated in CTNNB1, HepG2 is advantageous for HCC models by similar genomic and transcriptomic profiles.

Certain cell lineages (e.g. placenta and yolk sac) fail to develop in embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC). Expanded potential stem cell (EPSC) exceeds current stem cell lines in that they can develop into any type of cell, owning a greater potential for development. Here, we used human EPSC to generate VIO with STEMdiff™ Hematopoietic Kit and hanging-drop methods. Human EPSCs first differentiated and aggregated to form embryoid bodies (EBs), which then became VIOs, encompassing hematopoietic lineages.

HCC is prototypically inflammation-driven, making its microenvironment, featured by the immune-rich contexture, an appealing target for immunotherapies. However, the immune landscape of HCC remains incomplete, limiting the development of corresponding cancer therapies. Here, the incorporation of HepG2 into VIOs can recapitulate essential features of the HCC's microenvironment: vascularization of the organoid provides oxygen and nutrients and removes metabolic waste, maintaining tumour survival and enabling metastasis; incorporation of functional immune cells simulates the inflammatory microenvironment, the analysis of which can provide deeper insights into the tumour-immune cell interaction, thereby facilitating the improvement of HCC-targeting immunotherapies.

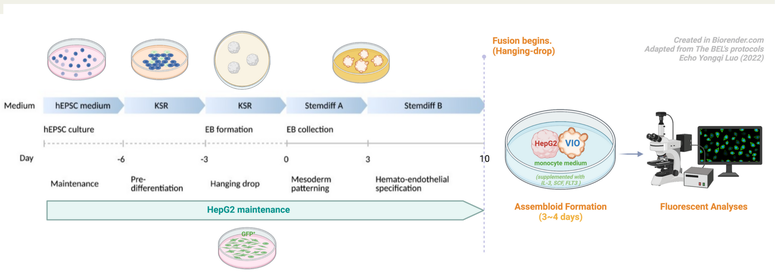
METHODOLOGY

1. Maintenance of cell lines (HepG2 and EPSC)

   (1) Subculture; (2) Routine medium change

2. Formation of human EPSC-derived VIOs
   (1) differentiation of human EPSC and formation of embryoid bodies
   (2) differentiation of embryoid bodies into VIOs

3. Formation of assembloids composed of Hep2G tumours and VIOs
4. Observation of cancerous-immune interaction in the assembloids under the fluorescence microscope



OBJECTIVE

1. To generate HepG2-derived cancerous liver assembloids that encapsulate essential features of tumor-immune cell interactions, using techniques including stem cell differentiation, organoid culture, and organoid assembly.

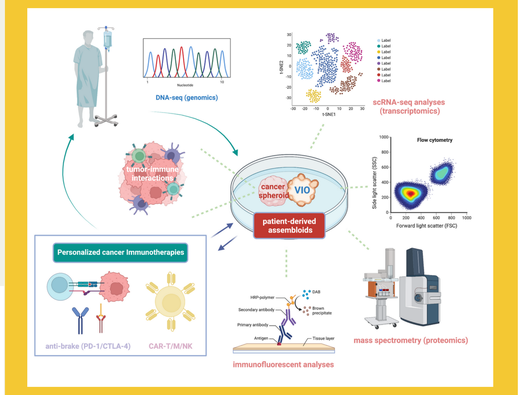
2. To evaluate the formation of assembloids and explore the interaction of cancer cells and immune cells using fluorescence microscopy.

RELATED LITERATURE

1. Kim, E., Choi, S., Kang, B. et al. Creation of bladder assembloids mimicking tissue regeneration and cancer. Nature 588, 664–669 (2020). https://doi.org/10.1038/s41586-020-3034-x
2. Giraud, Chalopin, Blanc, Saleh et al. Hepatocellular Carcinoma Immune Landscape and the Potential of Immunotherapies. Front. Immunol.(2021). https://doi.org/10.3389/fimmu.2021.655697

DISCUSSION

Future Directions



1. **[Patient-derived assembloids]:** Patient-specific assembloids are a promising platform to validate personalized medicine, in terms of efficacy (response) and safety (toxicity), and to target suitable participants for novel immunotherapies, thus supporting the development of cancer treatment in the early setting of clinical trials.
2. **[More comprehensive analyses]:** Several levels of information remain unraveled: (1) omics: genomics (by DNA sequencing), transcriptomics (by RNA sequencing), and proteomics (by Liquid Chromatography-Mass Spectrometry); (2) spatial distribution of certain molecules that can be visualized by immunohistochemical staining. Integrative multi-omics approaches identify molecular markers of cancer development and reveal multiplexed intercellular and intracellular interactions, which have the potential to become targets of future immunotherapies.

RESULTS

1. Generation & characterisation of vascular immune organoid

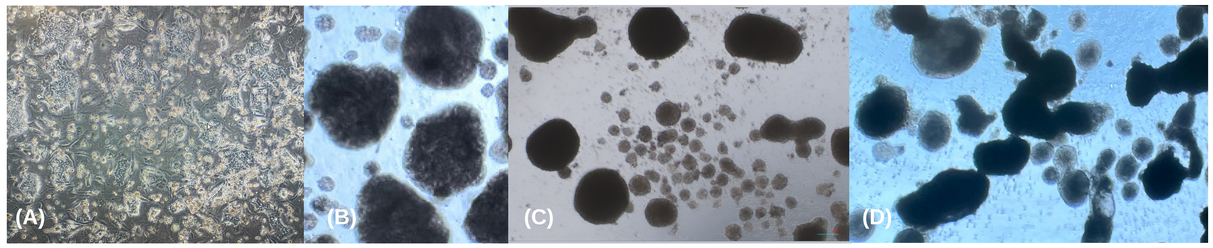


Figure 1 | Development of VIOs. (A) EPSC; (B) EBs (day 0); (C) EBs in medium A (day 1, mesoderm patterning); (D) EBs/ VIOs in medium B (day 10, hemato-endothelially differentiated). Hematopoietic cells and endothelial cells existed in VIOs, which could further differentiate into immune cells and vascular structures.

2. Generation & characterisation of assembloids

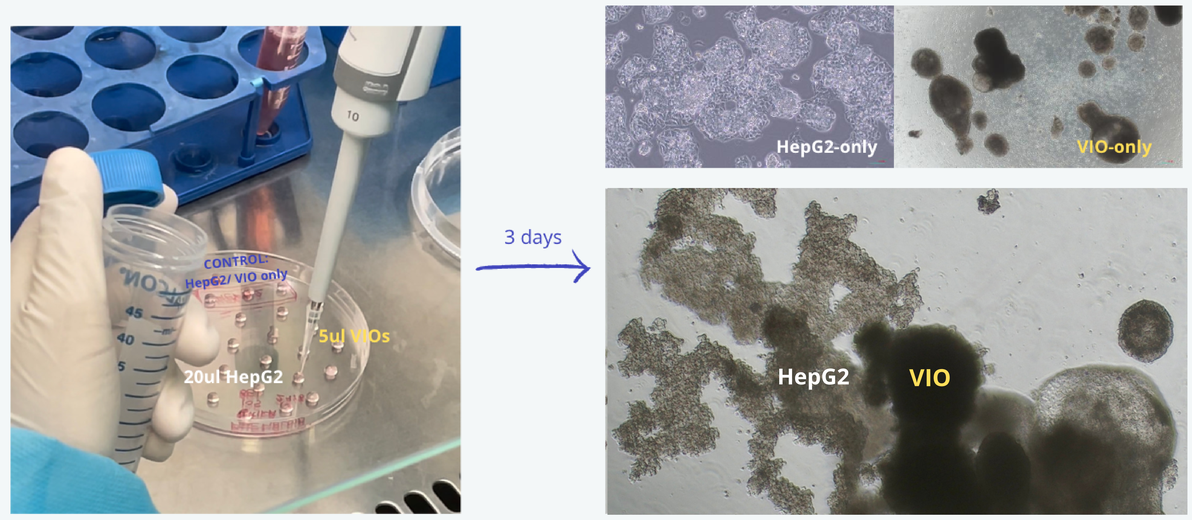


Figure 2 | Assembly of VIOs and HepG2. Left: diagram of assembly strategies (incorporation by injection, followed by hanging-drop aggregation); right: above are control groups, shown below is the assembloid.

3. Fluorescent analysis of immune-cancer interactions

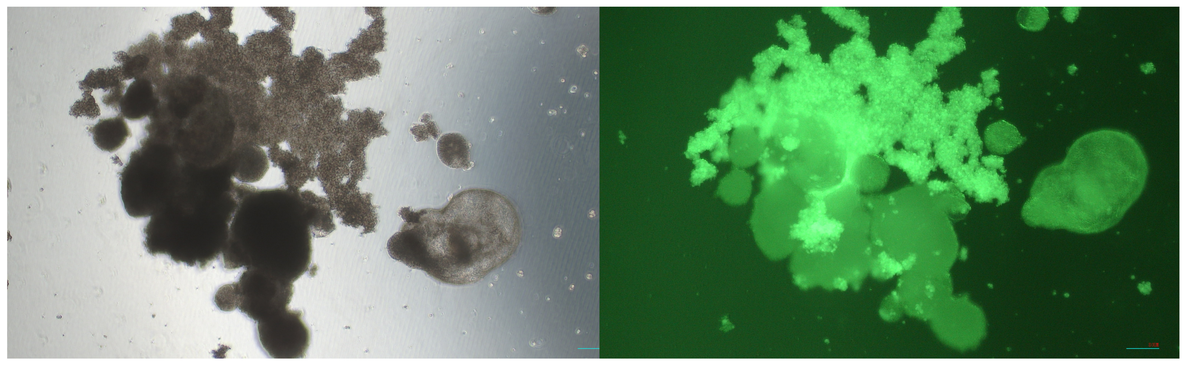


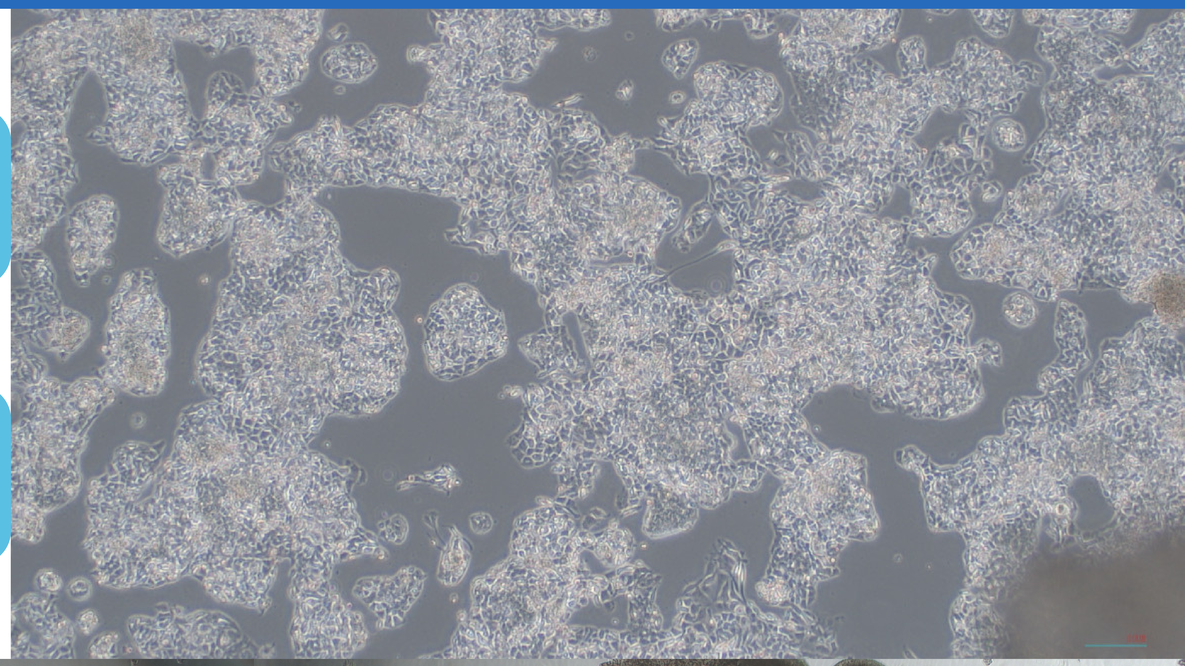
Figure 3 | Morphological characteristics of HepG2-VIO assembloids under the fluorescence microscope. Only HepG2 tumours were tagged with green fluorescent proteins, which made them distinguishable from VIOs (darker). The HepG2 tumours were fused with the VIOs, and some evaded into the centre of vIOs.

CONCLUSION

To summarise, we have created a pilot protocol of HepG2-VIO assembloid formation, further optimisation of which can more faithfully recapitulate the in vivo tumour microenvironment, leading to the establishment of an in vitro model ideal for immunotherapy.

SUPPLEMENTARY MATERIALS

S1: HepG2 cell line - GFP+



S2: Heterogeneous results of the assembly