Generation of Multicellular Liver Tumor Spheroids and its Application in Drug Screening

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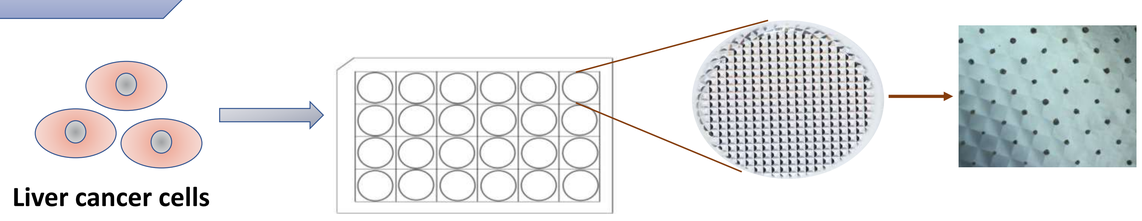
Background

Multicellular tumor spheroid is a new model of 3D culture to mimic the real solid tumors. The traditional 3D culture protocol has been established using one of the tumor cell lines. However, these tumor spheroids lack of heterogeneity of cell population, such as stroma cells and immune cells, in real solid tumors. In liver, there are macrophages, endothelial cells, and fibroblasts. Therefore, we propose that the multicellular tumor spheroids will be much better to mimic liver tumor in vivo.

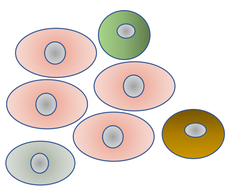
Methods



certain culture condition

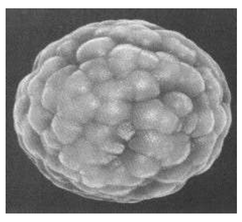
- Medium: with 5% PL or 10% FBS
- Oxygen concentration: 5% hypoxia or normal oxygen
- Liver cancer cell line: HepG-2 or HUH-7
- Matrigel

Collect cancer cell spheroids → CCK-8, RNA/protein analysis to confirm culture conditions



Cancer cells macrophages, endothelial cells, and fibroblasts

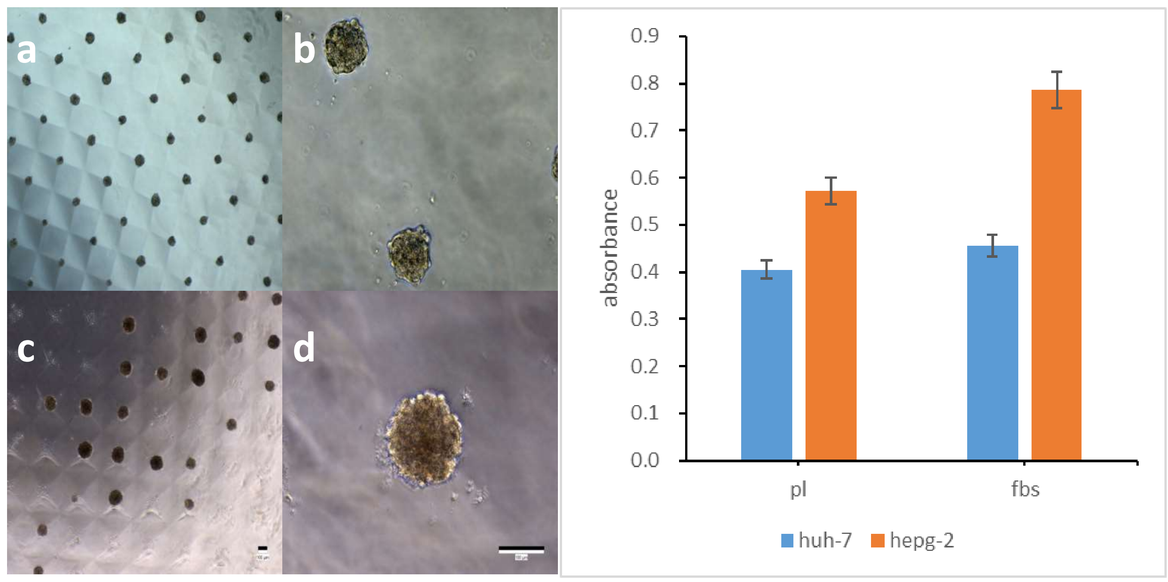
Co-culture →



MCTs

→ physicochemical property analysis → Drug screening

Result



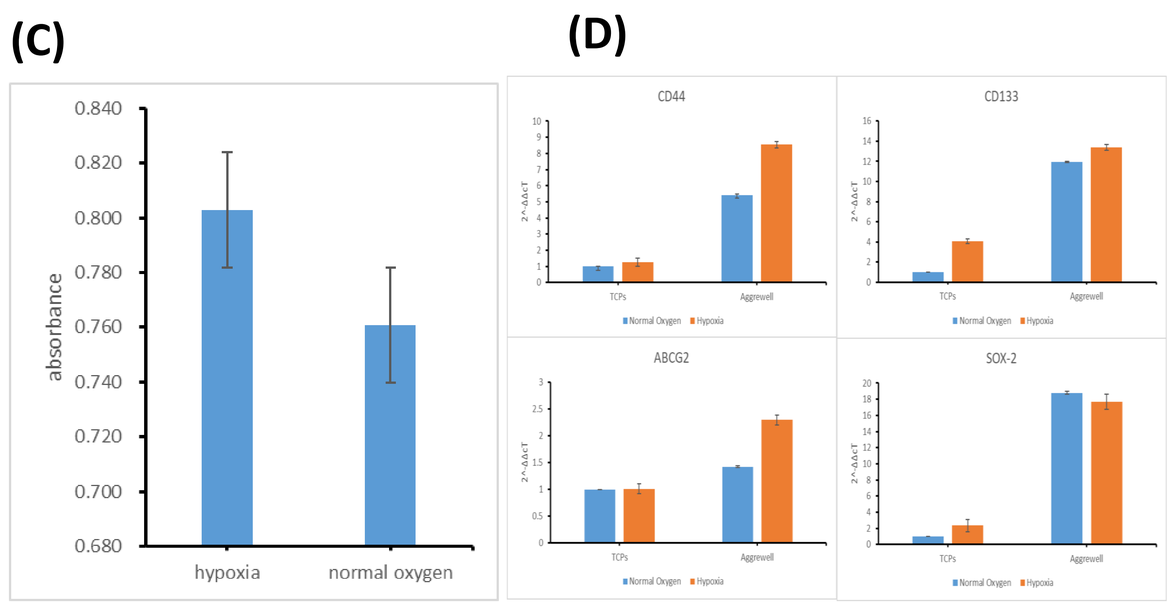


Figure 1. (A) Images of the MCTs cultured on porous agarose scaffold after 5 days. (a) 5%hypoxia, 10%FBS, without Matrigel (4× magnification) (b) 5%hypoxia, 10%FBS, without Matrigel (20× magnification) (c) 5%hypoxia, 10%FBS, with Matrigel (4× magnification) (d) 5%hypoxia, 10%FBS, without Matrigel (20× magnification) (B) The CCK-8 result of HepG-2 and HUH-7 cells cultured in the medium supplemented with PL and FBS respectively after 5 days. (C) The CCK-8 result of HepG-2 cells cultured in two oxygen concentrations (normal oxygen and 5%hypoxia) after 5 days. (D) The results of qPCR detection of cell spheres cultured in two oxygen concentrations (normal oxygen and 5%hypoxia) four target genes were CD133, CD44, ABCG2 and SOX-2.

Current data shows when HepG-2 cultured under the condition of DMEM with 10% FBS and 5% hypoxia, the cell spheroids can express more cancer stem cell genes, drug resistance genes and stem cell genes, which shows its potential in drug screening. Meanwhile, the experimental result also suggests that Matrigel may has positive effect on the establishment of cell spheroids.

Conclusion

With the improvement of culture methods and the addition of co-culture systems, we expect that MCT will be able to reproduce more of the physiological characteristics of the tumor in vivo; for example, stable expression of hypoxic inducible factor (HIF), loss of DDR protein, increased glycogen synthesis and glycolytic activity. The MCT model is an undeveloped technique that can improve our understanding of subpopulations within tumors and can help us better understand and target the adaptation of hypoxia to tumor cells. With increased interest in therapies targeting metabolic pathways, DDR proteins, and contextual synthetic lethality, the MCTS model could play an important role.

References:

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