比較2D細胞培養和3D腫瘤球的免疫毒殺差異

Differences between 2D cell culture and 3D Tumoroid culture for Human CIK effective assay

A6

基礎組-醫師

[1] Joseph Sergio Kyler(蘇吉歐), [1] Kamal Lawrence Andrew(安倫斯), [1,3] Yang,Sheng-Nan(楊生湳), [2] Bing-Sian Lin(林秉賢), [2] Meng-Han Tu(涂孟韓), [2] Chung-Wei Yu(余仲薇), [2] Cyuan-Ya Yang(楊荃雅), [2] Hsing-Tung Yeh(葉星彤), [2] Hsin-Ni Tsai (蔡欣霓), [2] Yu- Wen Kao (高郁雯), [1,2] Li-Feng Liu(劉麗芬)

[1] School of Medicine(後醫系), [2] Department of Biological Science and Technology(生科系), I-SHOU University(義守大學)；[3] E-DA Hospital(義大醫院)

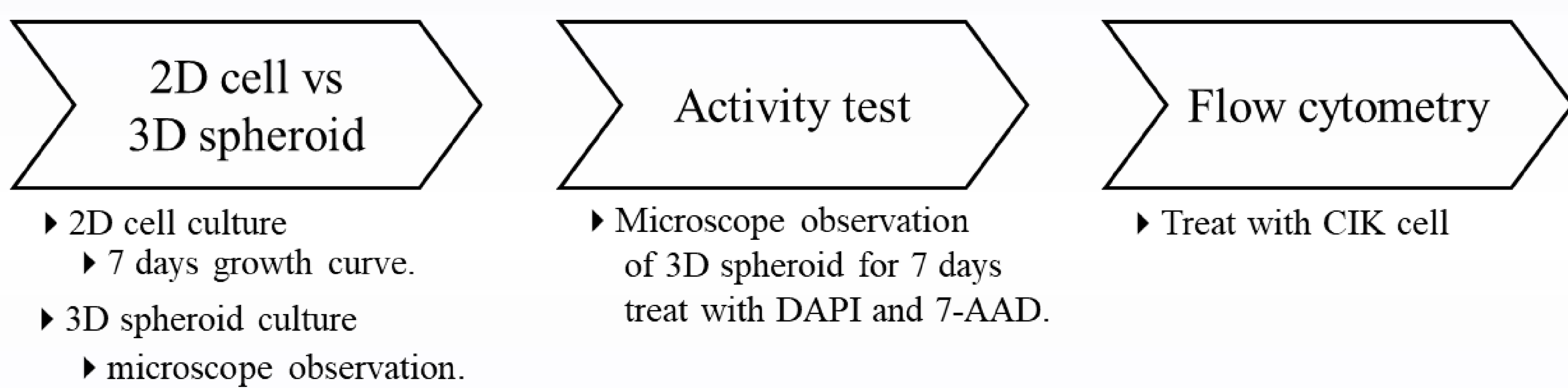
Abstract

Current research shows that cancer cell culture models are shifting from two-dimensional to three-dimensional, and 3D cell models are regarded as an important tool to reflect the real cancer cell microenvironment in pharmacokinetic and tumor microenvironment studies. In this study, various cell lines were used to study the differences of cell growth in between 2D and 3D culture systems, we found 3D culture model had a longer incubation time and a significant cell growth rate rather than 2D culture model. In 3D culture system, not all of cancer cell lines can growth in a sphere structure, the mouse fibroblast 3T3 and Human RCC 786-o cells were used to study the human CIK activities on 2D culture and 3D culture systems with a low affinity 96-well U-bottom. Microscopically, CIK cells target the oncolytic mechanism of renal cell carcinoma 786-O tumor-like tumors as well as interactions in vivo, such as CIK attachment and invasion, making the tumor less compact. Here, we show that 3D culture models of cell line tumors are more suitable than 2D cell cultures as a tool for therapeutic development in the field of immuno-oncology.

Introduction

3D cell models can learn more into cancer cell biology, is a convenient models for high-throughput drug screening, because they are low-cost and technically simple. And, it emulation is better than 2D cultures[1、2] at modelling tumor attributes such as hypoxia[3], dormancy and drug resistance. Although 2D cell culture models have proven valuable, compared with 3D tumoroid culture models, whether in simulating in vivo cell-cell interactions, intracellular roles, structural features, or metabolic or growth environments[4], 3D tumoroid models are closer to the real situation of clinical cases that can accurately represent the response rate of immune attack to tumor microenvironment in vivo. Here, we used mouse fibroblast 3T3 and Human RCC 786-o cells to study the human CIK activities on 2D culture and 3D culture systems with a low affinity 96-well U-bottom. 3D tumoroids.

Methods



Results

Depending on the characteristics of the cells, the effect of the cells forming the 3D tumoroid is also different. In Fig.1, various cell lines were used to study the differences of cell growth in between 2D and 3D culture systems, we found 3D culture model had a longer incubation time and a significant cell growth rate rather than 2D culture model. In 3D culture system, not all of cancer cell lines can growth in a sphere structure. In Fig. 2, the mouse fibroblast 3T3 and Human RCC 786-o cells were used to study the tumoroid formation by day's observation, In Fig. 3, 7-AAD and DAPI were used to study the cell viability of 7-days cultivated tumoroids and there was no death cells observed. In order to study the functional effective of tumoroids for the drug sensitivity evaluation, the differences of human CIK activities on 2D culture system and 3D culture with a low affinity 96-well U-bottom system were study. In Fig. 4, we found that the oncolytic targeting of 10-folds of CIK cells to leukemia K562 was 35±2% and targeting to 2D culture of 786-O renal cell carcinoma was 14.9±1.8% after 4 hours incubation. Oncolytic targeting of CIKs on 3D culture of 786-O tumoroids had shown a high condense CIKs attachment on the surface of tumoroids and a significant 3D structure dissolved by high efficient CIK penetration into tumoroids.

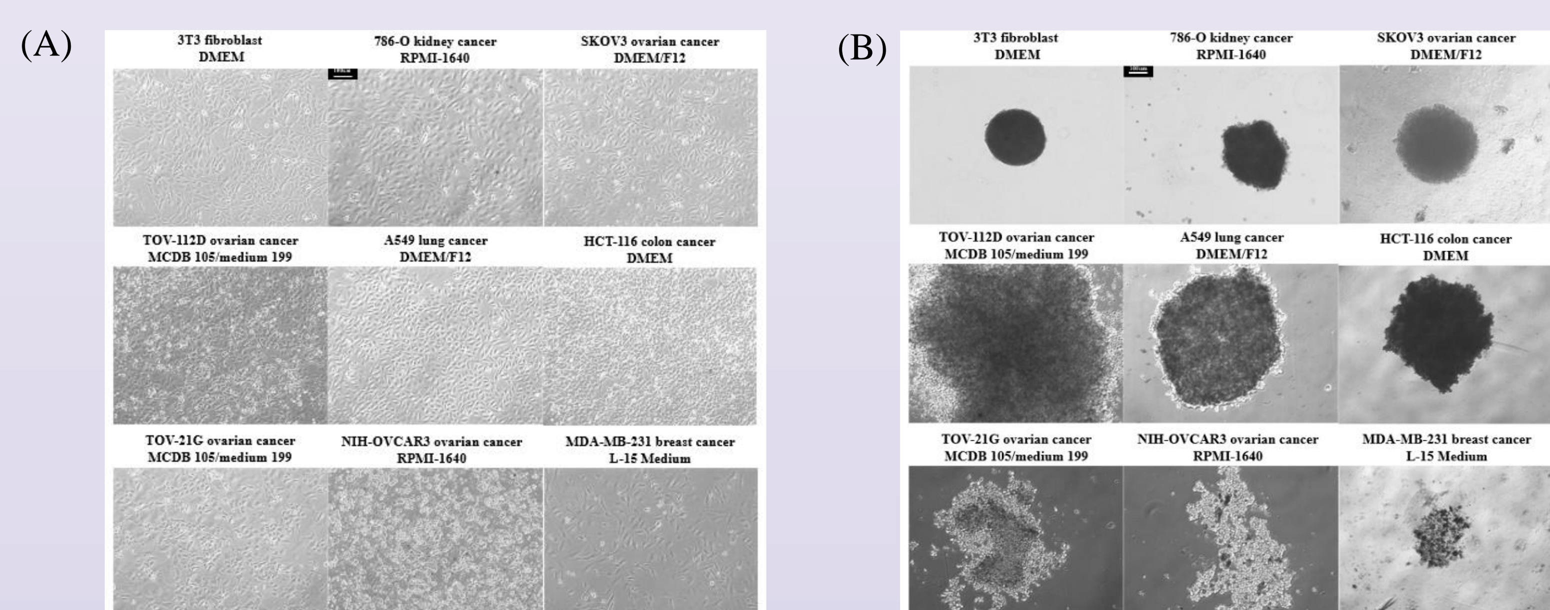


Fig. 1 Cell growth observation of Mouse fibroblast 3T3, Human RCC 786-O, Human ovarian (SKOV3, TOV-112D, TOV-21G, NIH-OVCAR3), Human Lung cancer A549, Human colon cancer HCT-116, and Human breast cancer MDA-MB-231
(A) Microscopically(SOPTOP-ICX41) documented 2D cell culture in 96 well (SAHD003-TCP011096-BX).(B) Microscopically(SOPTOP-ICX41) documented 3D tumoroid at U bottom(CLS7007-24EA).

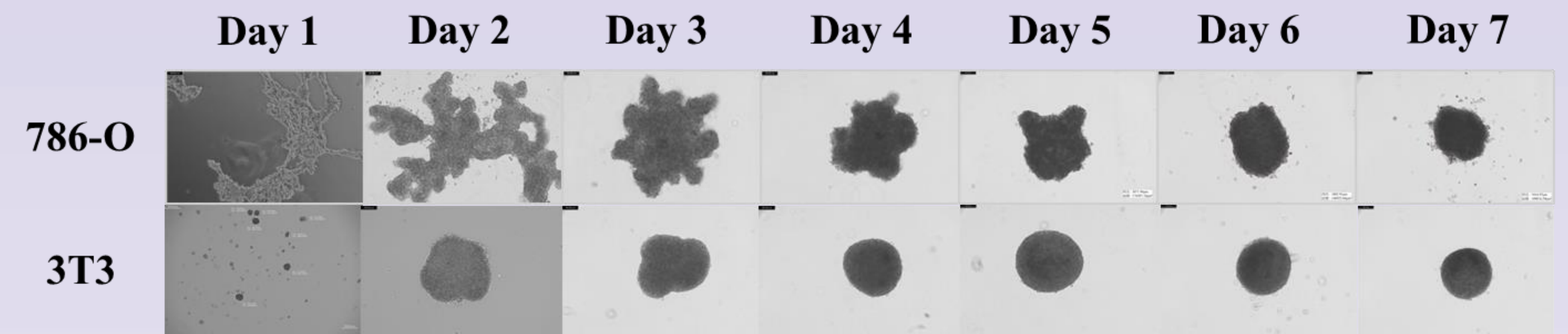


Fig. 2 786-O and 3T3 cell line 7 days 3D cell tumoroid.
(A) The 786-o and 3T3 cell culture seeding 8x10³ cells by U bottom(CLS7007-24EA), and then the cell morphology was determined using an microscope (SOPTOP-ICX41). 786-O cell tumoroid structure was loose from day 1 to day 3, and the structure was compact from the day 4 to day 7, and began to condense into a sphere;3T3 cell tumoroid formed small non-agglomerated spheres on the day 1, and condensed into 3D tumoroid with a solid structure from the day 2 to day 7.Scale bar=100 µm.

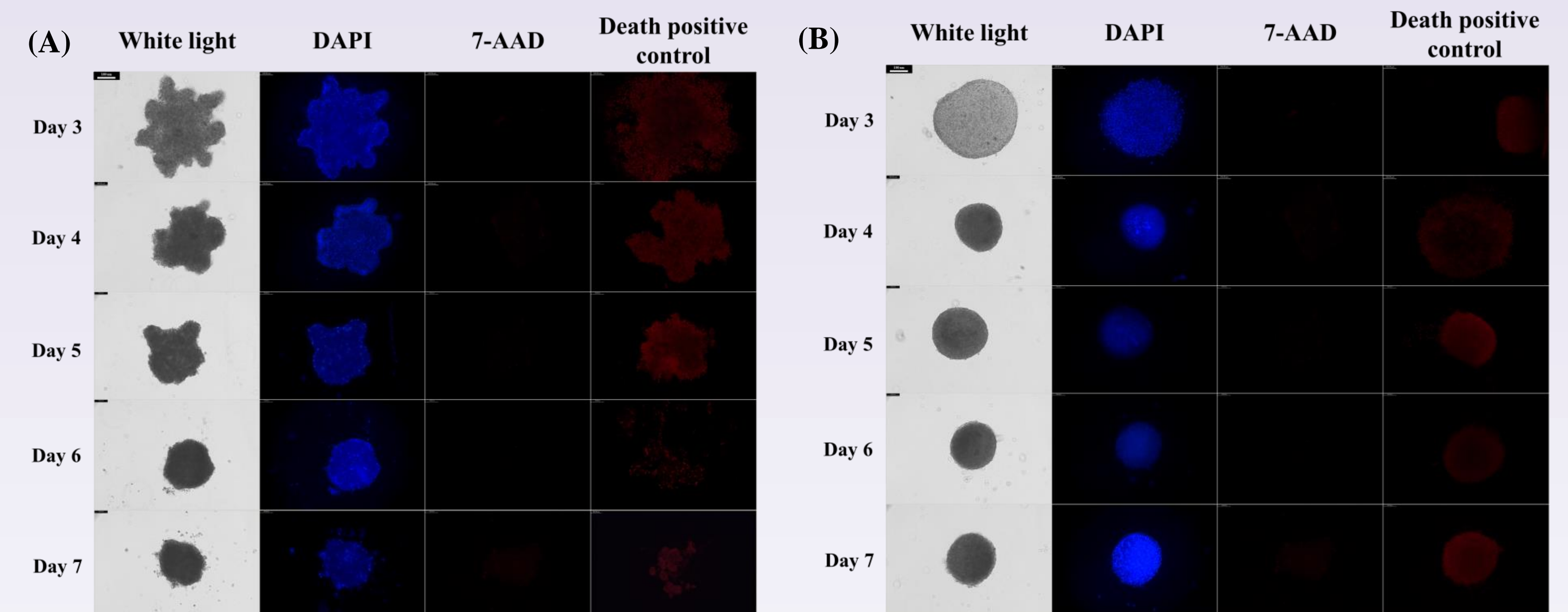


Fig. 3 786-O and 3T3 cell line 3-7 days 3D cell activity.
(A) Spheroid of the 786-O cell (8x10³ cells/drop) with 7-AAD, DAPI and Death positive control(cell culture 56°C water bath 30 min with 7-AAD), and then the cell morphology was determined using an optical microscope (SOPTOP-ICX41). (B) Microscopically(SOPTOP-ICX41) documented spheroid of the 3T3 cell (8x10³ cells/drop) with 7-AAD, DAPI and Death positive control(cell culture 56°C water bath 30 min with 7-AAD), and then the cell morphology was determined using an optical microscope (SOPTOP-ICX41).

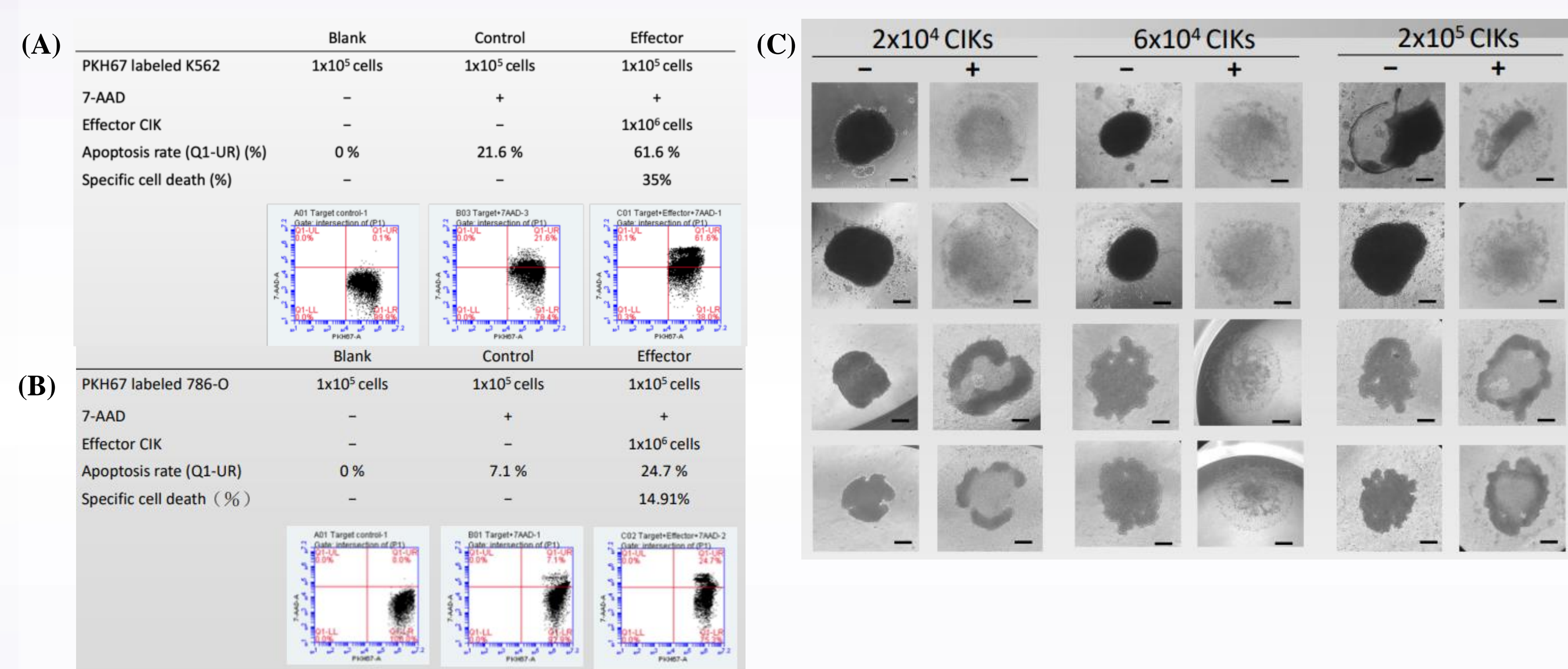


Fig. 4 Flow Cytometry-Based Cytotoxicity Assay for the Assessment of Human CIK Cell Activity and Micrographs CIK cell responds on Renal cell carcinomas 786-O tumoroid.
Flow Cytometry-Based Cytotoxicity Assay for the Assessment of Human CIK activity on(A) leukemic cell line and (B) tumoroid by Flow cytometer (BD Accuri™ C6 Plus).
(C) Micrographs of 2x10⁴ CIKs, 6x10⁴ CIKs, and 2x10⁵ CIKs activity on Versus Renal cell carcinomas 786-O cell by optical microscope (SOPTOP-ICX41). Scale bar=100 µm.

Discussion & Conclusion

A increasing number of studies demonstrate that 2D culture systems severely alter cellular phenotypes and physiology [6,7]. This could partially explain why only 16% of drugs developed based on results in 2D systems find success in phase II and phase III clinical trials, with cancer therapies representing a substantial proportion of the failures. Spheroids are convenient models for high-throughput drug screening because they are low cost and technically simple yet better simulate tumor attributes like hypoxia [3], dormancy, and drug resistance than 2D cultures [1,2]. In the research, the 786-O cell line has achieved remarkable results. It is hoped that in the future, the 3D model of other cell lines can be established by adding collagen, which can be applied in the research of drug testing and help the development of new drugs.

References

1. Drost J., Clevers H. Organoids in cancer research. Nat. Rev. Cancer. 2018;18:407–418.
2. Przepiorski A., Sander V., Tran T., Hollywood J.A., Sorrenson B., Shih J.H., Wolvetang E.J., McMahon A.P., Holm T.M., Davidson A.J. A Simple Bioreactor-Based Method to Generate Kidney Organoids from Pluripotent Stem Cells. Stem Cell Rep. 2018;11:470–484.
3. Pampaloni, F., Reynaud, E. G. & Stelzer, E. H. The third dimension bridges the gap between cell culture and live tissue. Nat Rev Mol Cell Biol 8, 839-845, doi:10.1038/nrm2236 (2007).
4. Kapałczyńska, M. et al. 2D and 3D cell cultures - a comparison of different types of cancer cell cultures. Arch Med Sci 14, 910-919, doi:10.5114/aoms.2016.63743 (2018).
5. Liu, X. et al. A Novel SimpleDrop Chip for 3D Spheroid Formation and Anti-Cancer Drug Assay. Micromachines (Basel) 12, doi:10.3390/mi12060681 (2021).
6. Arrowsmith J., Miller P. Phase II and Phase III attrition rates 2011–2012. Nat. Rev. Drug Discov. 2013;12:569.
7. Mestas J., Hughes C.C.W. Of Mice and Not Men: Differences between Mouse and Human Immunology. J. Immunol. 2004;172:2731– 2738.

Acknowledgements

This work was supported in part by grants from the E-DA Hospital (EDAHS111041, EDAHS111042), Taiwan.