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Short Communication

FBS *vs.* ACM: How should Human Cells be Cultured?

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ABSTRACT

A recent study showed that the traditional FBS-complemented culture technique selectively supported mesenchymal stromal cells in patient-derived cancer cultures, while An Autologous Culture Method (ACM) better-preserved the original, biological properties of an individual's tumor. This commentary article further discusses the possible applications of ACM in cancer research and clinical use.

Keywords: Fetal bovine serum; Patient-derived cancer culture; Autologous culture method

DESCRIPTION

Serum is commonly used as a supplement to basal growth medium in cell cultures. Fetal Bovine Serum (FBS) is the most commonly used type of serum because of its high concentration of embryonic growth-promoting factors [1-3]. FBS-complemented culture medium serves as a source of amino acids, proteins, vitamins, carbohydrates, lipids, hormones, growth factors, inorganic salts and trace elements. It also improves the pH-buffering capacity of the medium and helps to reduce shear stress. However, in a recent study using 4-5 patient-derived cancer samples, the FBS-complemented culture condition mainly supported mesenchymal stromal cell growth, rather than epithelial cancer cells [4].

Differences in cytokine productions and drug responses were also found between the two culture techniques4. Additional studies found that patients' PBMCs (peripheral blood mononuclear cells), which were cultured side-by-side in FBS-complemented (10%) and ACM (autologous serum), also presented different morphologies (unpublished data). The PBMCs were isolated from thirteen patients who had lung, stomach, breast or esophagus cancers. After six days in culture, large round cells appeared in ACM, while fibroblast-like cells dominated in FBS (Figure 1). Immunohistochemistry indicated that both of these cell types belonged to the macrophage family. However, FBS cultures induced CD163+ M1 cells, while ACM resulted in iNOS+ M2 cells (Figure 1). The above data suggest that FBS – the fundamental supplement that scientists depend on for laboratory experiments – might have a negative influence on the retention of cells' original biological properties, and could also cause unwanted cell-types to proliferate in culture wells.

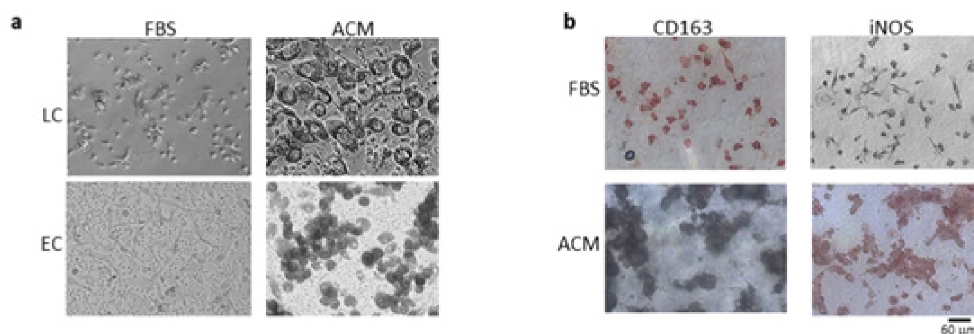

Figure 1: Morphological differences in patient PBMCs between PBS and ACM cultures. (a) Two representative culture images of PBMCs from Lung Cancer (LC) and Esophagus cancer (EC) Patients. PBMCs were seeded into wells in the same amount, and side-by-side cultured with FBS-complemented (10%) medium or autologous serum. Six days later, fibroblast-like cells dominated in all FBS culture wells, but many round cells appeared in all ACM cultures. (b) Immunohistochemistry (IHC) stain for an EC sample. Cover slips were inserted into PBMCs culture wells. After six days, cells were fixed with 4% formaldehyde inside the culture wells, and then the slips were removed for IHC stain. CD163 was positive in FBS-cultured cells, and iNOS was positive in ACM cultured-cells.

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Cancer researchers generally prefer techniques that can sustain cell cultures for longer times and support multiple passages, so that experiments can be easily repeated. Pharmaceutical applications further require high throughput for industrialization. To achieve these goals, cloned cancer cell lines (e.g. NIH60), combined with commercial culture media formulations, are commonly used in cancer research and anti-cancer drug development. However, this approach overlooks the key issue of patient individuality. A tumor's native characteristics-including its differentiation stage, local and systemic body fluid biochemistry, and cell-cell/cell-matrix communications in its in vivo microenvironment – are disregarded when artificial, uniform, standardized culture conditions are used. We believe that this is the fundamental reason for the disparity between laboratory results and clinical outcomes, when such cultures are employed in pre-clinical drug testing assays. The differing results of culturing PBMCs in FBS and ACM should also be cause for concern with regard to tumor-lymphocyte interaction assays in co-culture systems, because this type of assay routinely employs FBS-complemented culture medium [5].

Patient Derived Tumor Organoids (PDTOs) have become popular for human cancer research and drug testing [6-10]. The critical aspect of organoid technology is the formulation of a culture medium containing the essential/specific growth factors. These growth factors are usually added into FBS-complemented cell culture media to form a 'conditioned medium', to promote the formation of organoids [11].

As shown in Figure 2, the tissue-like structures that formed in ACM cultures resembled 'organoids'; morphologically they looked similar to the structures that originate from PDTO methods6-8. However, the 'organoids' that formed in ACM did not require any additional cytokines or growth factors; cells from patient-derived samples survived and organized themselves in autologous cultures. ACM 'organoids' contained not only cancer cells, but also stromal cells. The apparent mechanism behind this phenomenon is that the autologous serum/body fluid provides virtually all the nutrients, hormones, cytokines/chemokines and growth factors that an individual tumor receives in its in vivo physiological condition. It is nearly impossible to duplicate such an individualized ecosystem using commercial products. The organoids that develop in FBS-based PDTO cultures are likely due to the added growth factors because, in the study summarized above, fibroblast-like cells dominated in the FBS-complemented 3D condition, regardless of the type of sample. But it is unclear how manmade in vitro environments, such as FBS-complemented culture for PDTOs, influence a tumor's response to a treatment option.

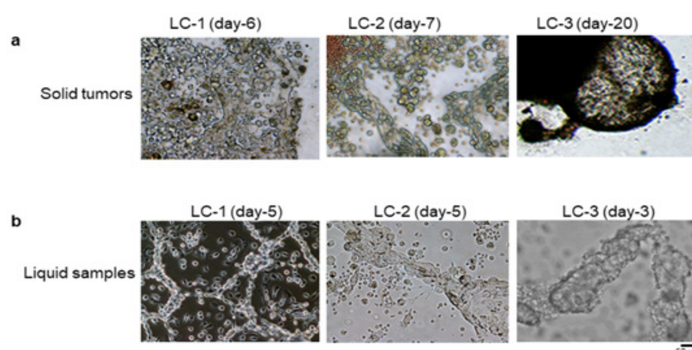

Figure 2: "Organoids" in ACM cultures. a. Samples from three resected lung cancers (LC-1 – LC-3). Tumor cells and matrix cells self-organized into structures that grew in culture wells (days in culture indicated). b. Single cell suspensions from three pleural effusions of a lung cancer patient (LC-1 – LC-3). Tissue-like structures formed in all ACM cultures (days indicated).

Because cancer organoid models retain some critical features of samples, they are expected to offer a revolutionary approach for drug screening, immunotherapy, and prognosis-related hallmark discovery. However, there is uncertainty as to whether drug effects in cancer organoids accurately predict what happens in patients6, [12]. The biggest challenge of most tumor organoids is that they usually comprise only epithelial cells andprogenitor cells, but not mesenchymal stromal cells such as fibroblast and endothelial cells. This shortcoming can be overcome by the ACM technique. Moreover, because self-organization and self-construction were observed as early as day-2 in ACM cultures of patient-derived tumor samples4, ACM could be a useful approach for improving accuracy in functional profiling [13], and in identifying dynamic changes in a tumor's response under drug-sensitivity/resistant assays.

CONCLUSION

Although promising, ACM culture is not a fully mature technique. At present, no comparisons between ACM- and growth factor-induced organoids have been performed, so it is speculative to assume that tumor organoids in ACM cultures will retain their native properties better than those in FBS media conditioned with growth factors. Additionally, the use of ACM techniques requires careful consideration of ethical issues-as well as very close access to, and coordination with, clinicians and patients. Nevertheless, ACM culture appears to have considerable potential to better-preserve the biological properties of patient-derived tumors. I believe this justifies further studies to understand its mechanism, and the research and treatment benefits it might provide.

REFERENCES

1. Bitesize Bio: A history of FBS: A timeline of cell culture media. 2020.
2. Jedrzejczak-Silicka, M. History of cell culture. IntechOpen, 2017.
3. Yao T, Asayama Y. Animal- cell culture media: History, characteristics, and current issues. Reproductive Medicine Biology. 2017;16: 99–117.

4. Tang Y, Xu Q, Yan M, Zhang Y, Zhu,P, Li X, et all. Autologous culture method improves retention of tumors' native properties. Scientific Reports. 2020; 10:20455.

5. Coutselinis A, Kentarchou P, Boukis D. Separation and identification of the insecticide "Endosulfan" from biological materials. Forensic Science, 1976;8:1-4.

6. ATSDR (Agency of Toxic Substances and Disease Registry). Toxicological Profile for Endosulfan. 2000.

7. EPA. Reregistration Eligibility Decision for Endosulfan, November. 2002.

8. WHO (World Health Organization). The WHO Recommended Classification of Pesticides by Hazard. 2005.

9. Yavuz Y, Yurumez Y, Kücüker H, Ela Y, Yüksel S. Two cases of acute endosulfan toxicity. Clin Toxicol (Phila),2007;45:530-532.

10. Lee I, Eriksson P, Fredriksson A, Buratovic S, Viberg H. Developmental neurotoxic effects of two pesticides: Behavior and neuroprotein studies on endosulfan and cypermethrin. Toxicology. 2015; 3:1-10.

11. Wilson W W, Shapiro L P, Bradner J M, Caudle W M. Developmental exposure to the organochlorine insecticide endosulfan damages the nigrostriatal dopamine system in male offspring. Neurotoxicology. 2014; 44:279–287.

12. Pradhan S, Pandey N, Phadke R V, Kaur A, Sharma K, Gupta R K. Selective involvement of basal ganglia and occipital cortex in a patient with acute endosulfan poisoning. J Neurol Sci, 1997;147: 209–213.

13. Krishnaveni M, Mirunalini S. Therapeutic potential of Phyllanthus emblica (Amla): The ayurvedic wonder. J Basic Clin Physiol Pharmacol. 2010; 21:93-105.