RESEARCH ARTICLE

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3D-reverts of HCT-116, MG-63, and SiHa Human Cancer Cell Lines – Promising Cancer Research Models

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Abstract

Objective: Human cancer cell lines have contributed immensely to cancer research. Advancements in cell culture techniques and contributions from modern biomedical engineering developments have enabled better utilization of such cell lines. There has been a major shift from the two-dimensional (2D) way of cell culture to the three-dimensional (3D) ways of culturing cells, which began about two decades ago. We propose an extension to this evolving trend in the form of 3D reverts (3DRs). **Methods:** 3D aggregates of three human cancer cell lines, HCT-116, MG-63, and SiHa, were obtained using agarose hydrogels as the matrix. 3DRs were obtained by introducing the floating 3D aggregates into scaffold-free culture units. These 3DRs were observed periodically, and images were obtained and analyzed for their culture characteristics. **Results:** The 3DRs of the three cell lines mimicked an explant-like features, with cells migrating out of the aggregates and attaching to the culture surfaces. Each cell line exhibited a unique pattern of migration of individual cells from their respective 3D aggregates to form 3DRs. The cells in the proliferative zone of HCT-116, MG-63, and SiHa aggregates showed single-cell mesenchymal-like, amoeboid-like and collective migration, respectively. The morphological features of the 3D aggregates of the cell lines used largely determined the type of cell movement exhibited for formation of the 3DRs. **Conclusions:** 3DR types of culture have not been well studied or described in detail previously. Although such cultures resemble 2D monolayers, the manner in which they develop differs among cell lines. Such 3DRs have potential as emerging models for in vitro cancer research experiments, especially for studies related to metastasis.

Keywords: Cancer cell lines- 3D cultures- 3D Reverts- in vitro models- cancer research

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Introduction

Experimental models available for cancer research have played a crucial role in understanding the complexities of cancer biology and developing effective anticancer strategies. Human cancer cell lines are invaluable for cancer research, their applications ranging from drug-induced toxicity testing to intricate studies of carcinogenesis and progression mechanisms [1, 2]. In-vitro models that utilize established cancer cell lines began by culturing them as the classic monolayers, the 2-Dimensional (2D) cultures. The advent of 3D culture systems combined with the development of microfluidic devices in which such 3D cell aggregates can be studied have greatly contributed to increasing the utility of such cell lines. This has enabled a better mimicking of the complex in vivo cancer conditions in vitro, leading to meaningful experimental outcomes with translational benefits [3]. The 3D cell culture models has marked a significant milestone as advanced experimental models offering more realistic in vivo-like environment in vitro. The primary advantage of 3D cell culture systems in tumor research is their ability to mimic the cell-cell interaction, nutrient and oxygen gradient, and complex tumor microenvironment more accurately forming multicellular spheroids or organoids. 3D cell culture systems have the capacity to recapitulate tumor heterogeneity. Moreover, 3D cell culture systems provide a platform for studying tumor invasion and metastasis with better clarity [4].

Advancements in culture methods can improve the experimental outcomes and also the scope of studies for which established human cancer cell lines can be utilized. One such possibility that we describe here is an extension of the "2D to 3D" cell culture techniques with the introduction to the 3D-Revert (3DR) cultures. 3DRs can be obtained by culturing 3D aggregates in a matrix/scaffold-free culture conditions as similar to that of growing the 2D monolayers. 3D aggregates obtained using

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matrix/scaffold-based culture systems can be harvested at their late-exponential or plateau culture phase and then transferred to scaffold/matrix-free culture system to obtain 3DRs. The 3D aggregates thus transferred behave as explant cultures with the cells contained within the aggregates beginning to migrate and form monolayer-like cultures. We propose that such 3DRs will have a different physiology compared to their 2D and 3D counterparts. The manner in which the entire cellular physiological processes differ spontaneously and significantly when 2D monolayers are grown as 3D aggregates can also occur when the 3D cultures and converted into 3DRs. Such a change in the cellular processes can be taken advantage of for certain focused studies to understand cancer biology in vitro. For example, the manner in which cells from 3DRs migrate out of the 3D aggregates can be utilized to study and analyze such movement hitherto described such as the single-cell and multi-cellular (group) types [5, 6]. Apart from analysing such gross cellular movement types, the finer single-cell movement types such as the amoeboid-like and mesenchymal type where focal adhesion of the migrating cells to the extra cellular matrix can be well studied using 3DRs. Another possible application of the 3DRs is their utility as models to study the Epithelial-to-Mesenchymal transition and the reverse which are essential for metastasis [7]. We have earlier reported results on the protein profiling and cytokine expressions of the 3DRs of a few human cancer cell lines in comparison to their 2D and 3D counterparts [8, 9]. In this study, we present a detailed description of 3DRs obtained from three human cancer cell lines HCT-116, MG-63 and SiHa with an aim to extend or enhance the utility of human cancer cell lines for a wider range of experimental applications.

Materials and Methods

The three human cancer cell lines HCT-116, MG-63 and SiHa were maintained as monolayers by standard methods in Dulbecco's Minimum Essential Medium (DMEM) supplemented with 10% FBS. Floating 3D cultures were obtained using 1% agarose hydrogels as the matrix. The 1% agarose hydrogels were prepared by dissolving the agarose in serum-free culture medium by heating till the entire agarose dissolved. 1 mL of molten agarose was added to each of the wells of a 24 well culture plate and was exposed to Ultra Violet light for 15 minutes inside a laminar flow cabinet. Such an exposure ensured sterility of the matrix along with an enhanced polymerisation of the gel. This was followed by the addition of 1 mL of DMEM supplemented with 10% FBS (complete medium) to the culture wells. The cells were harvested from confluent flasks of all three cell lines. The cell suspension was collected after trypsinization and was centrifuged at 1000 RPM for 10 minutes. The cell pellets were gently suspended in complete medium and cell count was performed using a Neubauer chamber along with viability using the trypan blue dye exclusion method. The seeding densities used for the HCT-116, MG-63 and SiHa cell lines were $4x10^4$, $3x10^4$ and $5x10^4$ cells/well respectively. The cultures were incubated at 37°C in the presence of 5% CO_2.

The floating 3D aggregates at the peak log phases of cultures were pipetted out without disturbing the agarose gel scaffold and transferred into scaffold-free culture plates. The cells were cultured in complete medium to obtain the 3DRs. Images of the cultures were obtained using Nikon ECLIPSE Ts2 inverted phase-contrast microscope under 4X and 10X magnification for observation and analysis.

Results

The 3DRs of HCT-116 cells behaved like an explant culture immediately after transferring the 3D aggregate. The cells in the proliferating zone started to migrate out as spindle shaped cells forming the monolayers. However, the 3DRs differed in the morphology from their 2D monolayer counterparts. The aggregate structure completely disappeared by the 4th day in culture and all cells were now adherent resembling a monolayer. (Panel 1A of Figure 1).

The cells in MG-63 3D aggregates started to move out and migrate to form 3D revert monalyer. These cells moved out in amoeboid like movement called 'blebs' and formed their own conlony of cells. The complete dissaggregation of the cells occurred by the 4th day. (Panel 1B of Figure 1).

Unlike to more compact 3D aggregates of the HCT-116 and MG-63 cell lines, the SiHa cells formed 3D aggregates with a more loosely packed clusters of aggregates with acinar and multiacinar-like morphology. The 3DRs of SiHa formed as clusters of cels moving in groups from the each of the acinar structures. Thus, 3DRs of the SiHa cells showed a more colony-like formation spread out over a larger culture surface. Such 3DRs survived for 5 days in culture. (Panel 1C of Figure1).

Discussion

The three cell lines used formed 3D aggregates with different morphological features. The differences were in the form of compactness and also the shape which can be related to the extent and differences in the extra cellular matrix secreted by each of these cell types which was fundamental for the 3D aggregate formation. The 3DRs formation process and their features of the three cell lines also showed an association with the distinct morphologies of the 3D structures for each of the cell line. Although the 3DRs of all the three cell lines declined or did not survive beyond the 4th day in culture, the process of 3DR formation was unique for each of the cell lines used.

The HCT-116 reverts forms epithelial like spindle shaped adherent cells as they moved out of the aggregate in a single cell migration pattern. The cells in the outer proliferative zone of the 3D aggregates started to migrate out and became adherent to the culture surface. Similar pattern of the 3DR formation was observed for the MG-63 cells. However due to the presence of several aggregates for the MG-63 3D cultures, several pockets of 3DRs were simultaneously being formed for this cell line. The formation of 3DRs of SiHa cell line was more dispersed and occupied a larger area of the culture surface due to the multiacinar nature of the 3D aggregates. Thus,

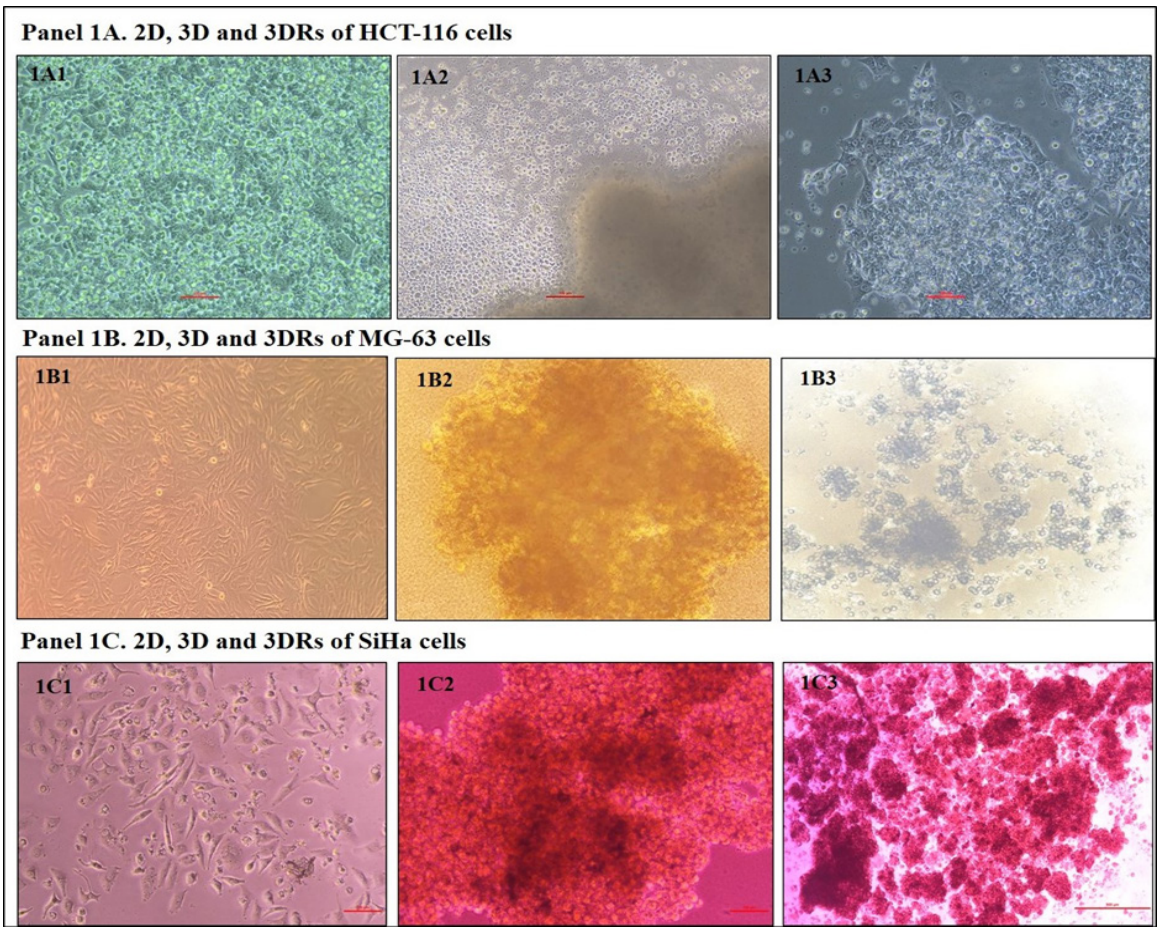

Figure 1. The 2D, 3D and 3DRs of the 3 Cell Lines had Distinct Morphological Features and Culture Characteristics. While the HCT-116 cells formed compact 3D aggregate structures, a less compact structure was seen for the MG-63 3D aggregates. The SiHa cells formed diffused aggregates with several clusters resembling acinar and multi-acinar structures. The manner in which the 3DRs formed differed for the three cell lines and appeared directed by the type of 3D structures of the respective cell lines. (Magnification: 10X; Scale: 100µm)

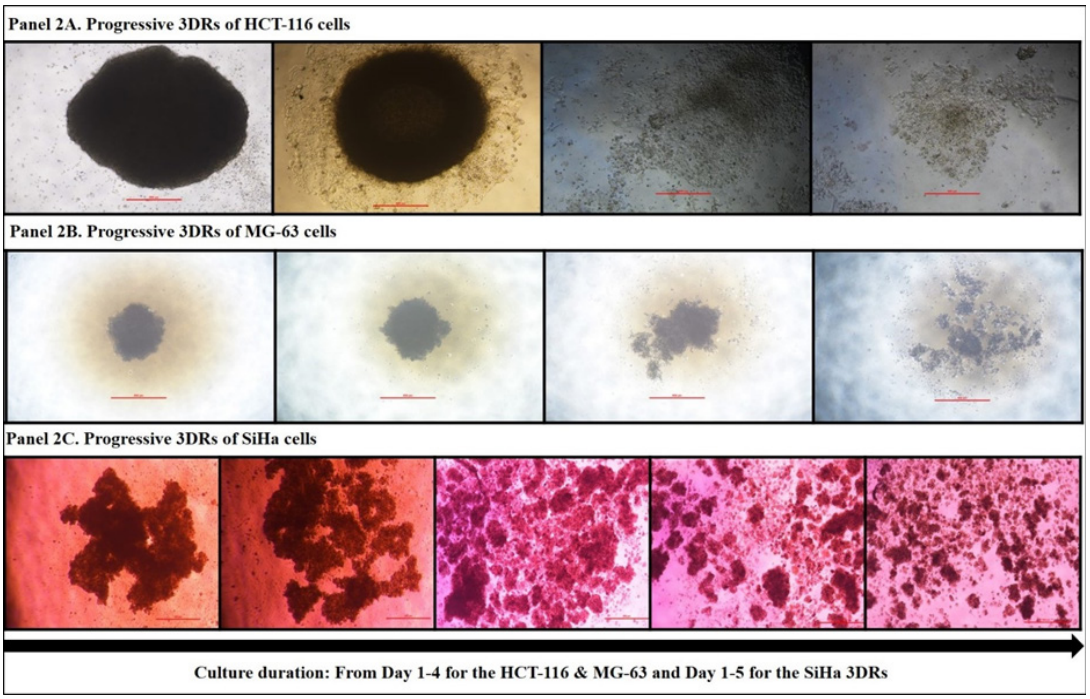

Figure 2. The Formation of the 3DRs of the 3 Cell Lines Showed a Similar Time-Dependent Pattern. However, the 3DRs of SiHa survived till day 5 while those of HCT-116 and MG-63 survived in culture for 4 days. (Magnification: 4X; Scale: 500µm)

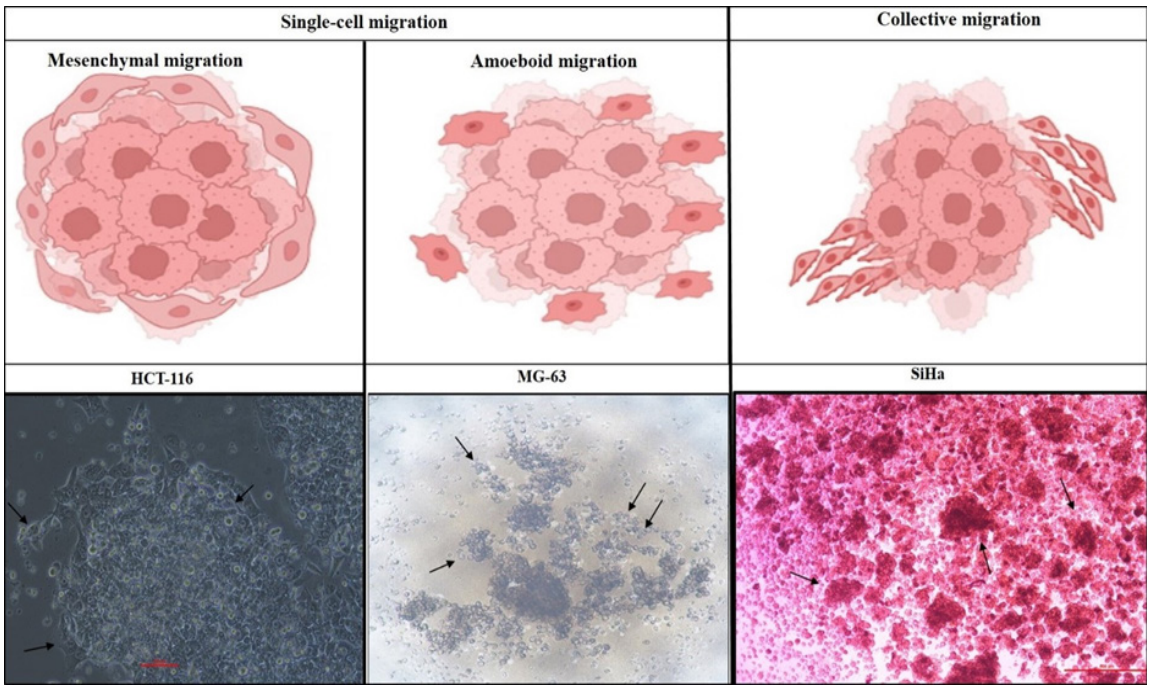

Figure 3. The Major Types of Cell Movements or Migrations Described in Literature Fall into Two Major Groups, the Single-Cell and the Collective Migration Types. Further, the single-cell moments can be of the mesenchymal and the amoeboid types. The 3DRs of HCT-116 were formed by the cells within the 3D aggregates showing a mesenchymal type of migration and adhering to the culture surfaces. The movement type exhibited by the MG-63 cells resembled the amoeboid type to form the 3DRs while the SiHa 3DRs were formed by a collective migration of cells present within the multi-acinar 3D structures.

the SiHa 3DRs were the most rapid in formation which is a reflection of the morphological features of the 3D aggregates of this cell line (Figure 2). The major types of movements observed during the formation of 3DRs for the three cell lines showing the "single cell" and "collective migration" as distinctive for each of the cell lines are presented in the Figure 3.

Enhancing the utility of human cancer cell lines for in vitro experiments have inherent advantages and we aim to present 3DRs as one more advancement of cell culture techniques. The strength of this model is in its primary utility to study cancer metastasis. The process of de-aggregation of 3D spheroids to form 3DRs might involve the mechanisms underlying Mesenchymal-Epithelial Transition (MET), an important feature for metastasis. The limitations of the 3DR models described is that they are emerging and more quantitative assays such as the cell migration & proliferation assays and molecular characterization especially those focusing on metastatic gene expressions are required for further validation. However, building on our previous reports and the details presented here, we feel 3DRs will be useful models for cancer metastasis.

Author Contribution Statement

All authors contributed equally in this study.

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Ethics statement

The described work is a part of the MSc Human Genetics Dissertation work of Ms. Raveena. D and the required Institutional Ethics clearance has been obtained prior to commencement of the experiments described.

Availability of data: There is no data available associated with this manuscript. Also, this study has not been registered in any registration datasets.

Abbreviations

2D, 2 Dimensional; 3D, 3 Dimensional; 3DR, 3 Dimensional Reverts; DMEM, Dulbecco's Minimum Essential Medium; FBS, Foetal Bovine Serum; MET, Mesenchymal-Epithelial Transition

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