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Approaches to Cancer Pharmacology and Cell Biology Using Three-Dimensional Models

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ABSTRACT

Cancer is a group of diseases involving neoplasia with the potential to invade or spread to other parts of the body. Cancer cells form a dynamic interrelation with their host. When taken *in vitro*, the specimen of human cancer has a high level of reduction and does not show heterogeneity or complexity. This suggests that the biochemical lap of cancer models representing the integrity which relates to a growing tumor developing in sedentary. An important feature is that all cells, tissues, and tumors grow in three dimensions. This three-dimensional models avail the treatment of cancer and secure a better scope in understanding the nature and complexity of a tumor as almost any of the newly prepared medications have failed to cure cancer in laboratory trials besides all the potent activities *in vitro*. Advances, types of 3D models used *in vitro*, further applications of 3D models and various other ecological factors are invigorated in this article. As these models are able to produce the impact on the study and securing the true nature of tumor biology in situ, a humongous work is still needed in order to present the biochemical laps which echo that of a tumor.

INTRODUCTION

In this area, we scrutinize the present status of *in vitro* and ex vitro cancer models, their complexity and heterogeneity in situ with respect to medicines. The recreation of 3D models *in vitro* has laid down the use of tumor cell lines in cancer drug discovery for solid tumor especially with the important fundamental of tumor stroma, the fibroblast. These not only change in size and scale but are indulged for shifts of cell culture. This was demonstrated by the application of bioreactor. The nature and persona of a tumor can be understood *in vitro* by the precession cutting of tumor slices. But this may hold some limitations on this podium. This query narrows the subject down to whether how many models can be approved for not only relating to drug discovery but also to the study of the disease *in vitro*.[1]

- ***Limitations of pre-clinical Cancer Models.***

The treatment of cancer has been affected very much by the influence of pharmaceutical industries and the availability of the drug which may provide the ultimate goal which may relate to the criteria studied for the treatment and targets set to achieve it. It was advocated that the assurance of drug discovery depends highly on the studies of the target testimony. This boundary can be encircled by the introduction of *in vitro* models and cell lines that attempts to summarize the pathology in a reductionist way.

Considering the drug and its area of interest, they follow the steps of the preclinical avalanche of drug discovery providing an aid for compound screening and refinement. This is again elaborated by introducing the cell lines into mice for further process. It comes to a realization that all the kinds of models used in the study have great limitations to express the complexity and heterogeneity of cancer. The failure of pre-clinical model incorporated *in vitro* is due to lack of novel cancer therapeutics as they have not produced much desirable potency in clinical trials.

- ***Cell lines: genotype versus phenotype relations.***

One illustration of a tumor is that it is referred to as ‘organ'. The problem arises as to how the cell lines growing on plastic surface interacts with the dynamics and complex stroma corresponding to the tumor [2]. Considering the heterogeneity in relation to the disease, the intra-tumor has been fulfilled by increasing the cell lines representing that pathology. On the other hand, single cell lines growing by suspending in agitated liquid media or on Petri dish

are considerably depended on the statistics unfolding to the sequence of their genome and express their range of mRNA, transcriptome.

The characteristic of cell lines' pathology is proved to be expressed by the correspondence in which they show deviation in their genetic pattern. The pharmacological reaction of cell line towards the agents is not clearly established. The limitation produced is that the biochemical circuitry of these cells is not fully learned. The relation between indicating torrents commenced by growth factors, cytokines and heterogeneous cell with the complex tumor in situ is yet to be explained. This issue is further preceded as the 3D model also has to express the above limitations. [3-4]

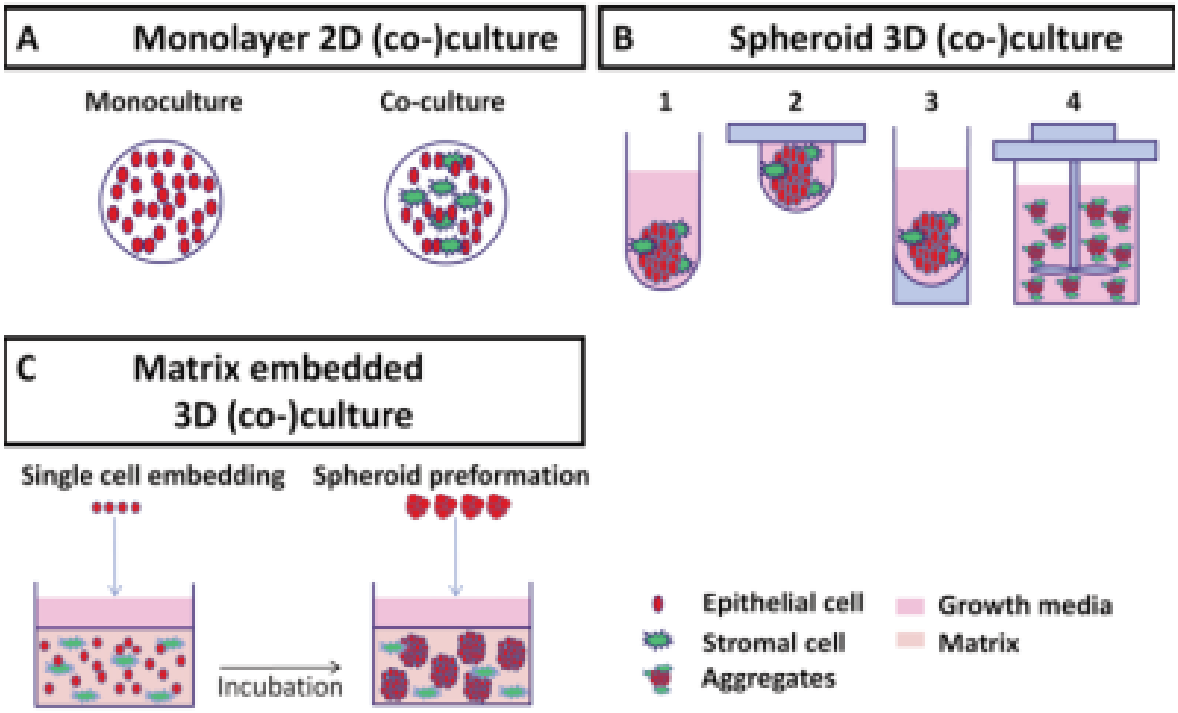

Figure: Two- and three-dimensional cell culture systems.

2D AND 3D CELL LINE MODELS

- ***Briefing the History of 2D Cancer Models***

In the earlier days itself, the relation between various techniques was analyzed. The *in vitro* cell culture and the introduction of cells in the mice had a very deficient clinical response. It was the cell culture, which was used to confront drugs in *in-vitro* techniques. Though these

techniques had limitations in similarity, they constructed a road to approach applications of chemotherapy in the treatment of human cancer only due to their dissimilarities.

In the 1970s, Hambarger and Salmon introduced colony forming assay. Even this advancement was a failure on a higher scale as it required new specimen from the patient frequently alongside with a low plating efficiency. In this technique, the new patient material was taken as a single cell solution in soft agar. Six tumor units- breasts, ovarian, melanoma, lungs, kidney and colorectal cultures that pass the quality controls confirmed that 20-35% of patients tested at sample size greater than 160 patients each. Four laboratories armed with the studies of NCI assessed its use in drug testing, a re-test of 79 compounds was done which were inert *in vitro* screening assay on 15 tumors each. The results showed that 14 compounds were active in at least two tumors. Considering clonogenic assay, chemotherapy was performed on 506 patients and return rate was found to be 27%compared to 18% for patients treated with empiric therapy. Just like the colony forming assay, the clonogenic assay also failed to produce desired and satisfactory results due to the constant supply of material from the patient. [5]

- ***Genomically Characterized Cell Lines Growing in 2D on Plastic***

Studying pathology, a very minute number of target molecules were able to produce a diligent effect. The desirable effect can be attended by genomically specific and distinct cellular models. By further examination on a larger scale established a fine correlation between cell lines whichever identified as considered to be responsive, their genotype and sensitivity marker established in clinical studies. The genetics of tumor cell lines were also used explain the activity of BRAF inhibitors vemurafenib towards BRAF mutant melanomas or mTOR inhibitor by raplogs induced AKT activation, teaching their low counter activity. These approximations fully eliminated the notion of a sromal compartment. Later the stromal matrix and tumor cells were studied which provided Straussmann and coworkers to put further the derivation of the some collaborated stroma-derived signals which may furnish sensitive tumor cell lines impervious to target drugs (eg: hepatocyte growth factor, HGF) which is produced by stromal cells returned vemurafenib sensitivity of V600E mutant cell lines. In addition, another attempt was made and six different growth factors effects were studied. The study further explained the resistance of tumor cell lines towards a set of kinase inhibitor that they were sensitive to. HGF, EGF, FGF, NRGI, and IGF all retrieved when used in combinations. In addition, expression of HGF was found in patients with melanoma

and certainly tallied with increased resistance to BRAF inhibitor. This adding complexity to 2D models may increase their prognostic power, particularly regarding signaling feedback loops leading to resistance.[6]

- ***Approach to the 3D Models***

We know that like everything, tumors also grow in the third dimension which may deny even the most advanced and sophisticated 2D models being used. These three-dimensional cultures are usually grown in bioreactors, small capsules in which the cells can grow into spheroids or 3D cell colonies. The 3D spheroids highly resembled avascular tumor nodule, micro-metastases and inter-vascular regions of large solid tumors. Principles of use of different techniques like hanging drop technique; stirrer-culture or stirrer-bioreactor etc. are some of the techniques which are used to prepare cells of the 3D culture. As these cells form clumps they discharge extracellular matrix components and unregulated proteins for cell-cell interactions. The spheroids hence produced showed a decreasing rate of oxygen levels, cell multiplication, and nutrition to center from periphery though they were firm and stable. In 3D spheroids, culture cells manipulate their sensitivity to cytotoxic agents such as 5-fluorouracil, cisplatin or doxorubicin. Even though being sensitive, they have a limited resistance towards these cytotoxic agents because of the cells which are present on the outer surface of spheroids becomes hypoxic and non-dividing. On the other hand, this could even be possible due to inappropriate drug delivery or even due to cell-cell interactions, the action of growth factors, expressing resistance markers like ABCC1 multi-drugs resistance pumps and also due to a production of extracellular matrix proteins. Medical drugs like phosphoinositide 3-kinase inhibitor PX-866 inhibits the growth of U87 glioblastoma, T47D breast and HCT116 colon cancer cells have shown effects in 3D spheroids though they were inert in 2D techniques. PX-866 inhibits PI3 kinase which controls development, proliferation, and survival of cancer cells. It has high power index in acting against a tumor and is now employed in clinical trials. 3D models have formed an excellent impact in clinical trials unlike their predecessor, 2D models as they are easy to predict and study the circumstances, easy to use and suitable for high throughput screening. Various techniques like fluorescence; luminescence etc. can be conveniently applied for the studying of cells. [7-8]

- ***3D And the Stroma***

Spheroid culture still misses the stromal compartment of a tumor. Examinations have exploited this affair by mixing tumor cells and fibroblast, fibroblast/monocyte suspension with tumor spheroids along with embryonic bodies or with other combinations effects of stromal cells and tumor cells have also been studied. For the treatment of various cancer types, considerable and distinguished 3D spheroids are required in order to match the degree of the relativity of resemblance of *in vitro* systems including growth rates, gene expression, signaling torrents and drug treatment responses. A comparison was established between 3D and 2D systems along with xenografts including a correlation with literature data available for the primary tumor. Comparison of 3D with 2D cultures expressed the up-regulation of E-cadherin, downregulation of vimentin, decreased expression of the proliferation marker MIB1, and increased expression of apoptotic marker caspase-3 in spheroids. The histology of the spheroids matched those of the original tumor in some cases, but there was a clear selection for the poorly differentiated high-grade histology. These models will establish a significant lead in drug discovery due to their various functional and analytical abilities. [9]

- ***Incorporating Extracellular Matrix:***

Models based on spheroids growing in the liquid medium still miss an essential component of tumor biology: the ECM. Pre-formed 3D spheroids embedded into or sitting on top of, a gel matrix could be analyzed for cell invasion and migration upon treatment with compounds. Using this assay, the HSP90 inhibitor 17-AAG and a phospholipase C (PLC) γ inhibitor could be shown to prevent dissemination on Matrigel™ (a laminin-rich basement membrane extract [BME] at concentrations below that to inhibit 50% of growth (GI50). However, the ECM is not just an inert matrix that provides the 3D scaffold for tumor cells to grow and invade but also plays an essential role in differentiation and maintenance of tissues, which may be missed in such hybrid systems. Moreover, the ECM has been shown to provide survival and drug resistance signals in cancer. β1 integrin signaling was shown to protect small cell lung cancer (SCLC) cells in clonogenic soft agar assays with added fibronectin from DNA damaging agents. Assays including ECM components may be performed in 2D, by coating plates with the respective proteins [10-11]. But due to the above-outlined advantages of growing cells in three dimensions, and the impact of matrix stiffness on cell growth and differentiation, embedding of the cells in the matrix is preferred. Matrix stiffness was shown to affect stem cell differentiation, tumor progression, invasion, and drug sensitivity.

Commonly used matrices in 3D cell culture area laminin-rich BME (also called Matrigel), purified from Engelbreth–Holm-Swarm (EHS) mouse sarcoma, composed mainly of laminin-111, collagen IV, and heparan sulfate proteoglycan, or collagen I. [9,12]

- ***Stirred culture vessels***

These include spinner vessels and computer-controlled stirred-tank bioreactors providing a dynamic stirred environment, overcoming mass transport and gas transfer limitations, as well as culture heterogeneity presented by other systems. Stirred vessels are scalable systems, with simple design and operation, extensively characterized hydrodynamically. Manipulation of parameters such as vessel and impeller design and stirring rate allow applicability to an array of cell types with distinct aggregative capabilities and sensitivities to shear stress, including a large panel of tumor cell lines. Importantly, stirred-tank bioreactors are highly flexible and can accommodate different 3D culture strategies, from cell spheroids to microcarrier/scaffold and microencapsulated mono and co-cultures presenting widespread potential. The main limitations of stirred-culture vessels are the hydrodynamic shear force-related cellular stress, promoted by stirring, and the high culture volumes associated with these systems (minimum of 50–80 mL). Microencapsulation and scaffold strategies can minimize cell exposure to shear-stress and improve microenvironment recapitulation by the accumulation of secreted factors and ECM components. [13]

- ***Microfluidic devices:***

Microfluidic devices, or micro-bioreactors, are efficient small-scale systems with precise control of cell microenvironment. Recent advances in microfluidic technology boosted the development of the novel *in vitro* drug screening methods compatible with high-throughput applications and the development of "organs-on-chips," composed of biologically functional tissue mimetics, in which inter-tissue interactions can be reconstituted. An array of designs has been proposed in recent years, with highly diverse innovative approaches to tumor microenvironment recapitulation. Recently, a microfluidic model to analyze the specificity of human breast cancer metastases to bone has been described. [14]

SUMMARY

Considerable progress that has been made in attempts to create *in vitro* models that are more representative of tumor complexity, particularly with cells that are surviving and/or growing in three dimensions with stroma, a number of caveats were highlighted. These complex *in vitro* platforms are surely an advance, recognizing that existing models have limitations. A systems approach is only viable if models of pathology and biology, used for validation, are representative of *in vitro* conditions. While very important strides have been made in modeling biology and pathology in animals, for example creating models to validate novel targets using genetically engineered mice, there is a continued need to develop and advance the use of appropriate *in vitro* models, such as those described. However, the community should be prudent about the use of novel platforms which often remain as "black boxes" and should contribute to their further characterization.

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