Int J Clin Exp Pathol 2017;10(2):842-852
www.ijcep.com /ISSN:1936-2625/IJCEP0040579

Review Article

The status and development of tumor microenvironment simulation platforms

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Received November 13, 2015; Accepted November 8, 2016; Epub February 1, 2017; Published February 15, 2017

Abstract: The tumor microenvironment, which is composed of tumor cells and non-malignant cells, plays a crucial role in malignant transformation, local invasion, distant metastasis and drug resistance. Reconstructing the tumor microenvironment in vitro has been used as an indispensable strategy to elucidate the mechanism of tumorigenesis, provide an early diagnosis and screen drugs. In the past few decades, several simulation platforms have been developed, including spontaneous cell aggregation, cellular scaffolding, the multicellular tumor spheroid model (MCTS), the rotary cell culture system (RCCS), and microfluidic devices. Using these systems, researchers have made significant progress in understanding the regulatory mechanisms of the tumor microenvironment and also in clinical research. These platforms can increase research efficiency, can help achieve individualized diagnoses and treatments and allow for high-throughput drug screening. In this review, we will introduce the current status of tumor microenvironment simulation platforms and their advantages and disadvantages. In addition, we further discuss their applications in their early clinical diagnosis and high-throughput screening of drugs, and their challenges and prospects in the future will be addressed.

Keywords: Tumor microenvironment, tumor microenvironment simulation platform, individualized treatment, drug screening

Introduction

The development and occurrence of tumors are not only associated with genetic alterations but also with the environment around the tumor [1]. This tumor microenvironment, which is composed of tumor cells and non-malignant cells, plays a crucial role in malignant transformation, local invasion, distant metastasis and drug resistance [2-4] via a so-called “seed (cancer)” and “soil (microenvironment)” relationship [5]. Through various biological processes, tumor cells can alter and maintain their own survival conditions to promote their growth and development [6]. Tumor stroma provides continuous support to carcinoma cells throughout different pathophysiological processes in response to molecular signals derived from carcinoma cells and other host cell types. Moreover, the structural architecture, which is called the extracellular matrix (ECM) and contains collagen, elastin and laminin, provides tissues with their mechanical properties and promotes communication between the tissue and cells [7].

In vitro mimicking of the tumor microenvironment is an indispensable methodology in both basic research and clinical studies. Over the past few decades, experts from different fields have designed various cancer models to reconstruct the tumor microenvironment [8, 9], ranging from two-dimensional (2D) cell culture systems to 3D cell culture systems, including spontaneous cell aggregation, cellular scaffolding, a multicellular tumor spheroid model (MCTS), a rotary cell culture system (RCCS), and microfluidic devices. Conventional monolayer systems cannot mimic actual tumor microenvironments and have various limitations in discovering new anticancer drugs and preventative treatments [9, 10]. The promising 3D cell culture systems

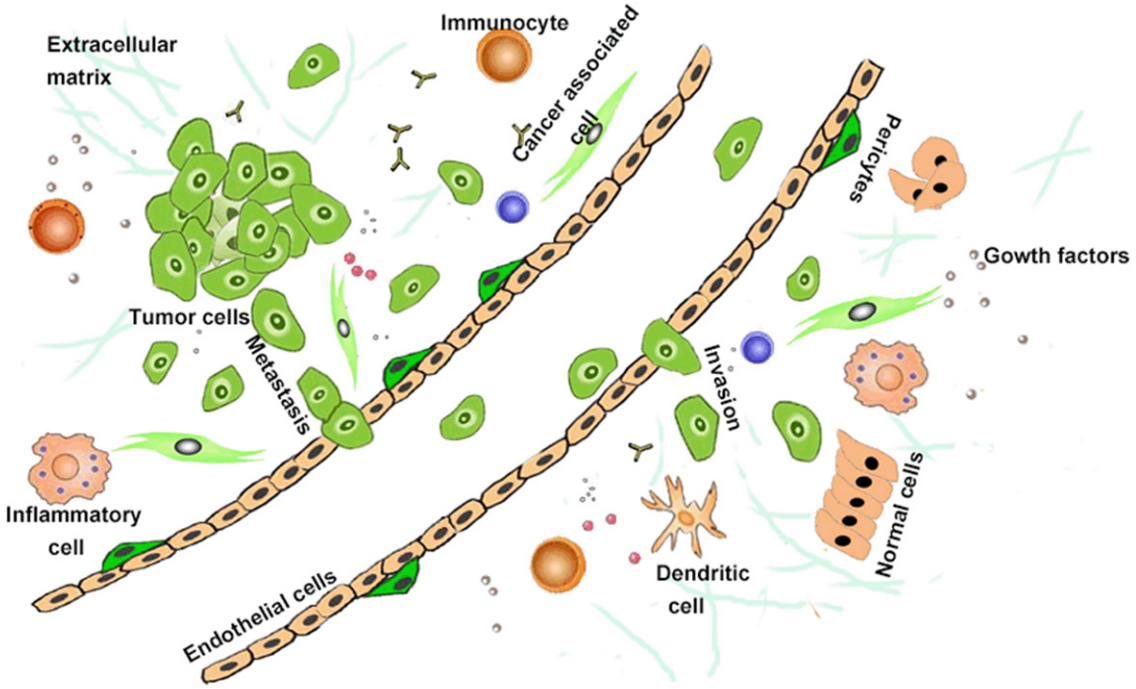

Figure 1. The model of tumor cell-microenvironment interactions. In the tumor environment, tumor cells maintain close contact with the activated cancer-associated fibroblasts and inflammatory cells through various growth factors and cytokines; at the same time, tumor cells enter the lymphatic system and blood circulation system to form circulating tumor cells (CTC). In the center of the tumor, regions of hypoxia form.

are commonly used as in vitro cell culture models and were developed to study tumor progression in vitro [11-13]. However, due to the limits of technology, there are still advantages and disadvantages to these systems. In this review, we discuss the simulation platform and its application in early clinical diagnosis and high-throughput screening drugs. In addition, challenges and future prospects are discussed.

The importance of the tumor microenvironment in cancer research

The tumor microenvironment plays an important role in tumorigenesis via mechanical or chemical actions. It is a complicated and dynamic system that involves in interactions among cancer cells; non-malignant cells; a number of stromal cell groups, including fibroblasts, immune and inflammatory cells, and endothelial cells (ECs); blood vessel cells; and networks of cytokines and growth factors [14, 15]. These interactions provide tumor cells with biochemical and biophysical cues [4]. The tumor microenvironment exhibits heterogeneity, with differing signals based on the types of tissues, differentiation stage, and pathological conditions [16, 17]. The crosstalk between the cancer cells and the tumor stroma is responsible for tumor progression and metastasis via a pyramid-like mechanism. Through autocrine, paracrine and hormonal signaling, tumor cells can alter and maintain their own survival conditions to promote their growth and development [6].

In the microenvironment, fibroblasts interact with cancer cells and are activated into heterogeneous cancer-associated fibroblasts (CAFs) or myofibroblasts [18] (**Figure 1**). CAFs remodel components of the ECM by increasing the production of ECM proteins and proteases. Additionally, they suppress the immune response by recruiting inflammatory cells (such as monocytes and macrophages) and modifying immune cell function to create a suitable environment for tumor growth [19, 20]. CAFs stimulate tumor proliferation, angiogenesis and metastasis through growth factors and cytokines, including vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-β), hepatocyte growth factor (HGF), plate-

let derived growth factor (PDGF), SDF-1, cyclooxygenase 2 (COX-2), and several interleukins (IL-1β, IL-6, IL-8) [21, 22]. VEGF secretion promotes multiple processes involved in angiogenesis, which facilitates cancer pathogenesis by supplying nutrients to enhance tumor growth [23]. In addition, pericytes, classical hallmarks of cancer, directly or indirectly contribute to tumor growth, metastatic spread, and therapeutic resistance [1].

The tumor microenvironment plays an important role in tumor metastasis and invasion. Tumor cells invade the surrounding ECM and assemble ECs to form new blood vessels or a similar new reticular structure of blood vessels called tumor vasculogenic mimicry, which is closely associated with the occurrence, development and metastasis of tumors and poor prognosis [24, 25]. Then, tumor cells enter the lymphatic system and blood circulation to form circulating tumor cells (CTC) [26]. In this process, cancer growth and vascularization are tightly regulated by hypoxia [27], cytokines [23] and a multitude of cell phenotypes and ECM components within the tumor microenvironment.

The importance of simulating the tumor microenvironment

Because the tumor microenvironment is a highly complicated system, further elucidation of the tumor microenvironment will increase our understanding of tumorigenesis and growth-promoting signaling pathways. Therefore, simulating the tumor microenvironment and developing appropriate model systems are indispensable method to study the physiology and biochemistry of tumor cells and address many questions. By simulating the tumor microenvironment, we can assess how a malignant state develops from a healthy tissue environment [28], qualify the role of the microenvironment in tumor migration, invasion and metastasis [29], gain insight into the expression of proteins under the regulation of microenvironment [30], and discover the spatial and temporal mechanisms of tumor angiogenesis and migration across the vascular system [31].

Constructing the tumor-resident environment to develop antitumor therapy is a useful method. Elucidating the metabolic pathways between cancer cells and antitumor immune cells could help guide cancer immunotherapy [32]. A better understanding of the relationship between epidermal growth factor (EGF) and the EGF-receptor could be used to inhibit the migration of breast cancer cells. Additionally, studying how tumor stroma affects tumor cell biological behavior could help design targeted therapeutic strategies [19]. Researching the relationship between the tumor and vascular compartments may facilitate prediction of patient prognosis.

Elucidating tumor metastasis mechanisms by reconstructing the tumor microenvironment will not only identify meaningful therapeutic targets [32] but will also be an important tool for predicting drug metabolism and toxicity in vitro. Presently, several cell-based models have been used to predict drug metabolism and toxicity to obtain more accurate parameters of treatment responses [33].

Approaches for simulating the microenvironment

Since Carl Jensen designed the earliest tumor model [34], in which he transplanted mouse sarcomas into healthy mice and measured tumor growth to estimate the vitality of the transplanted cancer, a large variety of tumor cell culture models have emerged, including the conventional monolayer technique, spontaneous cell aggregation, cellular scaffolding, the MCTS, the RCCS, and microfluidic devices. Although each model has its own set of advantages and disadvantages, the best choice is often a model that is simple and has clinical relevance for human patients [35].

Conventional monolayer technique

Two-dimensional cell culture techniques, such as 2D Petri dishes, 2D multi-well plates or 2D glass slides, have the advantages of convenience, operability, low cost, widespread application and ease of culturing a single cell. These in vitro method are commonly used to study malignant tumors and analyze antitumor drugs [36-38]. Cells cultured in these systems could be exposed directly to nutrients, oxygen or specific environments [39]. Two-dimensional cell culture systems could closely mimic in vivo physiological conditions [8]. In addition, different generations of cells can be preserved over long durations. Thus, we can not only study the

Table 1. Comparison between different materials

Material	Advantages	Disadvantages
Silk fibroin	Good biocompatibility [48]	Low hydrophilic property [49]
	Good biological adhesive [50]	Slow degradation [50]
	High tensile strength	
Collagen	High elasticity [51]	Lack of flexibility [51, 52]
		Low tensile strength [52, 53]
Fibrin glue	Small antigen [54]	Rapid degradation [54]
	Low immunogenicity	Poor mechanical strength [54]
	Good biocompatibility	
Chitosan	Easy processing [56]	Low solubility [55, 56]
	Inexpensive	
	Good biocompatibility [55]	
Alginate	Strong adsorption [57]	Instability [57]
	Porous	Resists degradation
Agarose	High hydrophilic property [58]	Cannot resist high
	Inert	Temperature [59]
	Stable	
Poly glycolic acid	Good biocompatibility	Low hydrophilic property [60]
	Mechanical strength [60]	Poor biological adhesive
Polyethylene glycol	Good biocompatibility	Cannot be degraded
	High mechanical [61]	
Poly (lactic-co-glycolic acid)	Good biocompatibility [63]	Poor biological adhesive [64]
	Mechanical strength	
	Controllability [62]	

response of the same generation under different conditions but also observe dynamic changes in different generations. In the past decades, cancer biologists, biomedical researchers, and oncologists have used 2D Petri dishes to study the complicated tumorigenic mechanisms of angiogenesis, invasion, and metastasis [40]. These models offer significant advantages for preclinical cancer drug discovery efforts given their simplicity and low cost.

The disadvantages of the 2D monolayer cultures used as in vitro models were uncovered gradually. In conventional 2D conditions, ECM components and the cell-to-cell and cell-to-matrix interactions, which are important for differentiation, proliferation and cellular functions in vivo, are lost [41]. Due to the lack of the ECM components as a structural architecture that supports and connects the cells and alters the organization and cell physiological activities, specific signaling between tumor cells and the molecular gradient, which is an important factor for cellular activities, are unavailable [9, 39]. Tumor cells grow in an adherent monolayer and lack a true 3D environment. The activities of these cells are limited. In addition, trials with these models did not provide information on the chemotherapeutic response mechanism. It is difficult to predict the effect of drugs in the body and study the effect of restriction by numerous extracellular barriers in the body that could otherwise lead to significant reductions in the infiltration capacity [9, 10]. However, these models have thus far been inadequate for discovering definitive cancer treatments. In addition, these models are unable to accurately simulate the true tumor microenvironment.

Spontaneous cell aggregation

A deeper understanding of the mechanism and development of clinical treatments for tumors have been hindered by using a traditional monolayer technique [42]. Since Bissell demonstrated the different behaviors of cancerous breast cells grown in 3D culture, 3D systems have served as the major in vitro cell culture model for studying tumor progression [11-13]. Early application of the cells in a 3D cell culture model is spontaneous cell aggregation [9]. In this method, tumor cells grow into spheroids or

other 3D forms spontaneously or upon induction by artificial substrates that induce cellular differentiation and maintain cellular function. Compared with 2D cell culture techniques, this system closely mimics the complex structures and organization of tissues in the body [43] where tumor cells reside histologically as multicellular tumor spheroids (MCTS) and provide biochemical as well as physical cues between the ECM and the basement membrane (BM) [44, 45]. This system could be used to co-culture different cell types, facilitating cell-cell interactions and the exchange of growth factors and other biological effectors; thus, these strategies expand research on the molecular mechanisms of adhesion, migration and invasion [9, 10]. Unfortunately, this method is limited to specific cell types and the cell interactions and group sizes cannot be controlled.

Cellular scaffolds

To closely simulate a 3D environment, biologists have employed engineered scaffolds to reconstruct the ECM and provide physical/structural support. An ideal cellular scaffold possesses the following traits: (1) good biocompatibility and does not cause inflammation and abnormal reactions in the body; (2) made of appropriate biodegradable material; (3) lacks immunogenicity and toxicity; (4) maintains cell morphology and phenotype, promotes cell adhesion and proliferation, and induces tissue regeneration; (5) conducive to the diffusion of nutrients. However, in fact, each material has its advantages and disadvantages [46, 47] as noted in **Table 1.**

MCTS

MCTS systems using in vitro tissue culture methods allow tumor cells to grow into 3D multicellular spherical structures by implanting tumor cells into a specific scaffold, such as a collagen scaffold [65], semi-solid medium (agar or agarose) [66], and liquid media [67], and culturing the cells to resemble the dimensional effects of the in vivo tumor microenvironment. The tumor cells within spheroids are in close contact or communicate with each other, and this strategy was proposed as a promising method for the maintenance of differentiated functions. Therefore, these systems can bridge the gap between 2D tissue culture models and animal cell culture systems in the field of studying tumor biology, interactions and drug responses. The major methods of preparing multicellular tumor spheres include the rotating culture method [68] and static culture method [67]. AccBased on their material properties, the systems can be divided into two primary groups: scaffold-based systems, a platform that can be used to investigate the effect of primary external physical factors on microspheroid growth and signaling [69], and non-adherent, liquid-based systems that allow for the exchange of medium to a certain degree [70].

Given their inherent properties, such as closely arranged cells, hypoxia, and heterogeneity [71, 72], these models have been viewed as ideal tools to provide new insight into phenotypic and cellular heterogeneity and micro-environmental aspects of in vivo tumor growth [70, 73]. These models have also been used to study microenvironment interactions, particularly intracellular signaling and other functional or cellular processes between exogenous ECM molecules and tumor cell receptors [74-76]. MCTS models can not only be used for elucidation of various mechanisms but have also produced important advances in response to radiation injury, cancer drug screening and drug discovery [65, 73]. Because the tumor sphere is similar to normal tumor tissues, it can be used to determine specific tumor tissue sensitivity to chemotherapy and radiotherapy, which can help identify new anti-cancer drugs and reduce side effects or drug resistance [65]. In addition, multicellular tumor spheres act as pathological cancer cells in the in vitro immersion attack model, which is useful for analysis of multiple factors [77].

Although these models provide significant advances in the simulation of the tumor microenvironment, some innate limitations still exist. The diffusion of oxygen and nutrients are limited by the spheroid model, which restricts the size of the spheroid [78].

RCCS

Many tumor cells fail to retain their specialized features and dedifferentiate when cultured under traditional 2D static cell culture conditions. To optimally induce shear force and turbulence, which are known to cause cell damage in cell culture, researchers attempted to maintain cells in suspension with various types of bioreactors [79]. However, all of these sys-

tems have disadvantages. National Aeronautics and Space Administration (NASA) first developed rotary cell culture systems to simulate microgravity, in which cells are cultured in a dynamic fluid suspension in liquid media mixed by minimal hydrodynamic forces [9]. RCCS have become a large-scale expansion of the cell culture system.

Compared with traditional methods, RCCS exhibit three distinct characteristics: (1) Tumor cells, gases and nutrients are evenly distributed. In a static training system, tumor cells often are limited to the bottom of the system, and the interior lacks a sufficient number of cells. Moreover, gas, nutrients and metabolic wastes are unevenly distributed, resulting in the accumulation of waste products and alterations in local pH. These changes may inhibit the normal tumor microenvironment and prevent the cells from obtaining sufficient nutrients, leading to slow growth or even stagnation [80, 81]. However, RCCS are dynamic systems that promote diffusion of oxygen and nutrients, discharge more metabolic waste, promote cell growth and have a uniform distribution of the tumor cells. (2) Centrifugation and stir are conventional methods to suspended cells in the Petri dishes. However, these generate shear force to damage cells. Thus, cells and tissues spend a considerable amount of time on repair, and tissue differentiation is hindered. However, RCCS use gravity-free, low-shear-force cell cultivation [82]. (3) In 3D cell cultures with inert material scaffolds, cells tend to attach to one another on the microcarriers to form complex 3D structures [82]. The following limitations are present: a complicated operation process, high cost and a lack of integration.

Microfluidic devices

Microfluidic devices, a breakthrough in simulating the tumor microenvironment, have profoundly affected our understanding of the tumor microenvironment and provided guidance for clinical treatment. These approaches were developed based on advances in micromechanics, microelectronics, biotechnology and nanotechnology. These approaches allow sample collection, reaction, separation and detection to occur in a basic operation unit at the submillimeter scale and to automatically complete the whole process analysis. Compared with traditional experimental technology, microfluidic devices have the following important characteristics: (1) These devices accurately simulate the microenvironment. Microfluidic devices create a 3D environment that more accurately reflects the human body using multiple cell types co-cultured with cytokines. Using new materials, we can even recreate the tumor microenvironment under hypoxic conditions [83, 84]. In addition, multiple types of cells can be co-cultured on the microfluidic devices. Thus, the signaling pathway or sequential changes between cells in vitro can be assessed [85]. (2) This technique allows biomedical research to be conducted in real-time and under controllable conditions. We can observe changes in various processes and obtain valid data in real time. In addition, we can combine required instruments into a microfluidic chip [86]. (3) This method requires a small amount of sample, which is indispensable for clinical research. Thus, we can diagnose diseases with small samples and reduce the cost of drug screening [87]. (4) This technique is flexible and portable, offering concentration of molecules in space and time [88, 89]. Therefore, this system may be useful for personalized diagnoses and personalized medicine based on drug toxicity screening and disease modeling for drug target discovery. These characteristics satisfy the demand of researchers for biochemical experiments, given that the characteristics of this methodology (i.e., a small amount of liquid, high-throughput, automation and particularly controllability) are lacking in gene and protein devices [87].

However, a multitude of challenges still exist: (1) Poor reusability, i.e., once polluted, the sample cannot be continually used in the study; (2) Failure to realize full automation; (3) Low throughput because the adsorption of antigen and antibody cannot be controlled under the high flow velocity; thus, we should limit the velocity of the fluid; (4) Lack of popularization, as this system is exclusively used in the laboratory and not currently used for clinical purposes.

Application of simulation platforms

What can these simulation platforms be used for? In recent decades, the diagnosis and treatment of tumors, drug research and tissue engineering have benefited from these platforms and a series of auxiliary equipment, including

fluorescent dyes, Western blot analysis, and polymerase chain reaction (PCR) assays. Based on the results from these platforms, personalized treatment and precision medical diagnoses were realized. Complex pharmaceutical processes have become simple and high-throughput with high content.

Cancer is a major cause of mortality. However, the leading cause of death in patients with tumors is CTCs [90-93]. Microfluidic devices can be used to detect and isolate the CTCs [94, 95], allowing for early diagnoses, individualized treatments and evaluations of prognosis [96]. In addition, microfluidic devices, which are inherently rapid and sensitive, have been used for point of care testing (POCT), referring to a portable mini test system outside the central laboratory that is close to the test object and offers timely results [97-100]. At present, the development of a personalized means of analysis is one of the important directions of microfluidic chip POCT research for illness monitoring and early diagnosis [101].

Although conventional in vitro platforms have made substantial contributions to screening drugs, these methods still have different limitations. Because 2D cell culture models are static states, as time progresses, these models cannot generate the mechanical or chemical stimuli (signaling molecules) that are normally present and simulate the complex internal environment in the body or the true extracellular mechanical environment [3, 102]. Currently in preclinical stages, 3D cell culture models have been proposed as promising in vitro methods to evaluate and predict tumor responses to chemotherapeutic agents [103-105].

The current trends in drug discovery screening require both high-throughput and high content. Thus, 3D cell culture technologies are naturally indispensable tool [106]. MCTS systems are employed for predicting dynamics, screening drugs and reducing the toxicity and side effects to normal tissues [107]. Microfluidic devices not only mimic complicated internal environment in vitro but also generate concentration gradients required for physiological activities [108] and high-throughput drug screening [109, 110]. This device would be a simple and convenient but high-efficiency tool to identify drug targets, study drug metabolism mechanisms and drug response, and assess drug genotoxicity and cytotoxicity for anti-cancer drug/agent discovery. Using microfluidic devices to study the major factors that affect the efficacy of the anticancer drugs has been previously reported [111, 112].

Conclusion

In this review, the role of the tumor microenvironment has been elucidated with various platforms. However, our current knowledge is just the tip of the iceberg. Many detailed mechanisms are uncharacterized. For example, how does the microenvironment affect tumorigenesis specifically? What are the key factors influencing the formation of tumors? Significant steps forward have been made over the past few years in basic research and clinical application. Two-dimensional cell culture techniques have allowed us to recognize the microscopic world of the tumor. Three-dimensional in vitro tissue culture models enrich or broaden our horizons. Clearly, the development and application of 3D tissue culture technology will be the hot spots in the future, especially in terms of miniaturization, integration and automation. However, complex production processes and high-throughput features are a major stumbling block to the use of these devices. Our hope is that with interdisciplinary collaboration, we will be able to design high-throughput equipment using 3D printing technology to simplify the production process.

Acknowledgements

We thank the Qingdao Institute of Biomass Energy and Bioprocess Technology of the Chinese Academy of Sciences for their technical guidance.

Disclosure of conflict of interest

None.

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