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Development of Tumor Models: From Mouse Tumor Models to Tumor-on-a-Chip

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Abstract. Cancer is currently one of the diseases with the greatest impact on human health worldwide. The development of anticancer drugs has always been a primary focus in the medical and pharmaceutical fields. Establishing models that simulate the human tumor microenvironment is a crucial aspect of preclinical trials for cancer drugs. For decades, researchers have been dedicated to developing models that increasingly resemble the true tumor microenvironment within the body, aiming to improve the success rate of clinical trials for anticancer drugs. The development of tumor models has gone through several stages, including two-dimensional mono-layer models, animal models, spheroids, organoids, and tumor-on-a-chip. This article focuses on the development from animal models, exemplified by mouse tumor models, to tumor-on-a-chip. It elaborates on the mechanisms, establishment methods, advantages, and disadvantages of both types of models. By summarizing, the article highlights the issues of clinical relevance and experimental reproducibility that have been the focus of tumor model development, the challenges of standardization, large-scale production, and cost reduction that need to be overcome in the future. And the importance of strengthening multidisciplinary cooperation and materials science and other related fields for the development of tumor-on-a-chip in the future.

Keywords: development of tumor model, mouse tumor model, tumor-on-a-chip, disease model.

1. Introduction

Cancer is one of the leading causes of death globally and poses a severe threat to human health. The global cancer burden is extremely high, with approximately 20 million new cancer cases and about 9.7 million deaths worldwide in 2022 [1]. The high incidence and mortality rates of cancer make the development of related drugs urgent, yet the success rate of anticancer drug candidates in clinical trials is very low. The overall success rate of cancer drugs from the first human clinical trial to eventual FDA approval is less than 4% [2]. A major reason for the failure of most anticancer drugs in clinical trials is the lack of preclinical models that can simulate the tumor microenvironment. Previous failures in drug clinical trials have raised higher standards for the construction of future tumor models.

Mice are good animals for establishing tumor models because they are low-cost, have short reproductive cycles, and are easy to genetically modify. However, animal experimental models face significant ethical issues, and many drugs that perform well in mouse models still have a high failure rate in human clinical trials. Thanks to the development of biomechanics, bioengineering technologies, and interdisciplinary fields, organ-on-a-chip technology has made significant advancements. Tumor-on-

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a-chip, in particular, involves the generation of 3D tumor models controlled by microfluidic devices, used to simulate important characteristics of tumor physiology.

This article uses mouse models and tumor-on-a-chip as examples to explain the mechanisms and advantages and disadvantages of both models, analyze the issues addressed during the development of tumor models, and discuss the challenges that need to be overcome for future tumor model development.

2. Overview of TME

Understanding the tumor microenvironment is essential for constructing tumor models. The tumor microenvironment (TME) refers to the complex and dynamic system surrounding tumor cells, which includes a variety of cellular and non-cellular components. These components interact with tumor cells to influence their growth, invasion, metastasis, and response to therapy. Understanding the TME is crucial for developing effective cancer treatments. Following content is the key component of TME [3]. First: Cancer cells: These are the primary cells that proliferate uncontrollably and form the bulk of the tumor. Second: Stromal cells: This group includes fibroblasts, immune cells (such as macrophages, T cells, and neutrophils), endothelial cells, and pericytes. They provide structural support and contribute to the biochemical environment of the tumor. Third: Extracellular matrix (ECM): The ECM is a network of proteins and glycoproteins that provides physical scaffolding for the cellular constituents of the TME and plays a critical role in cell signaling. Fourth: Blood vessels: The formation of new blood vessels (angiogenesis) supplies the tumor with oxygen and nutrients, facilitating its growth. Fifth: Immune cells: These include both tumor-promoting and tumor-suppressing cells. The immune landscape of the TME can influence the effectiveness of immunotherapies. Sixth: Signaling molecules: These include cytokines, growth factors, and hormones that mediate communication between cells within the TME.

3. Mouse tumor models

Mouse tumor models are widely used tools in cancer research and preclinical drug testing. They involve establishing human or murine tumors in mice to study the onset, progression, and treatment of cancer, serving as a common method to simulate the tumor microenvironment.

3.1. Subcutaneous and orthotopic mouse tumor models

The simplest model is the subcutaneous tumor model, where human or murine tumor cells are initially cultured in vitro (xenograft models) and implanted subcutaneously into the flank of mice [4]. Tumor growth is monitored by regular measurements of tumor length and width using calipers to calculate tumor area, allowing for cost-effective and highly reproducible experiments. In contrast, orthotopic models involve transplanting tumor cells into specific organs of mice to mimic their primary growth site [5]. These models more accurately simulate the tumor microenvironment and its interactions with the host. Despite their widespread use in preclinical drug development, they fail to replicate the complexity of cell origins and carcinogenic multistage processes found in the original tumor environment, thereby providing reliable preclinical data without conclusively demonstrating clinical efficacy.

3.2. Chemically induced models

This method involves inducing tumors in mice by exposing them to carcinogenic chemicals, thereby simulating the mechanisms and development of human cancer models [6]. Depending on the research goals, appropriate carcinogens such as DMBA [7] and MCA [8] are selected, commonly used in breast cancer and glioma studies, respectively. Carcinogens are typically administered through subcutaneous or intraperitoneal injections or oral gavage, with tumor occurrence sites and growth monitored periodically. This method can mimic the effects of environmental carcinogens on humans, aiding in understanding the mechanisms of cancer onset. Although the use of carcinogens is relatively straightforward, tumor induction requires a longer experimental cycle, posing significant mutational burdens.

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3.3. Genetically engineered mouse models (GEMMs)

Genetically engineered mouse models involve modifying the mouse genome using gene editing technologies to study gene functions and disease mechanisms [9]. Models are typically established through gene knockout, knock-in, or transgenic methods. Knockout models use CRISPR-Cas9 [10] or homologous recombination to delete specific genes, studying the impact of tumor suppressor genes on cancer development. Knock-in models involve inserting exogenous genes or mutant genes into specific loci of the mouse genome to simulate specific gene mutations seen in human cancers. Transgenic models insert exogenous genes, often oncogenes, into the mouse genome to study the effects of gene overexpression on tumor development. GEMMs more accurately mimic genetic variations and disease progression in human cancers, providing better predictions of clinical responses to drugs. However, their high costs for establishment, maintenance, and breeding, coupled with lengthy experimental cycles, limit their application in drug development.

3.4. Patient-derived xenograft (PDX) models

PDX models involve transplanting human tumor tissues into immunodeficient mice to establish clinically relevant tumor models. Tumor tissues are obtained from patient surgical resections or biopsies, and immunodeficient mice serve as hosts to prevent rejection of human tumor tissues by the mouse immune system. Tumor tissues are transplanted subcutaneously or orthotopically into mice, with regular monitoring of tumor growth and progression. PDX models retain the tissue architecture and microenvironmental characteristics of the original tumor, demonstrating high clinical relevance and reflecting the heterogeneity within tumors [11]. They aid in studying treatment responses across different tumor subtypes. However, PDX model establishment and maintenance are costly and time-consuming, and the use of immunodeficient mice does not fully replicate the interactions between human tumors and the immune system, limiting some clinical predictive value.

4. Tumor-on-a-chip

The tumor-on-a-chip is an advanced biotechnological technique that combines microfluidic technology with tissue engineering. Its purpose is to simulate intra-tumoral and microenvironmental conditions in vitro using microfabricated devices. This specialized application of organ-on-chip technology aims to model the growth, invasion, metastasis of tumors, and their interactions with surrounding tissues and the immune system within an organ-like structure [12]. It provides insights into the pathophysiology of human cancers.

4.1. Design and components of tumor-on-a-chip

4.1.1. Microfluidic technology

The tumor-on-a-chip constructs three-dimensional tumor models on microfluidic chips to mimic the growth environment of tumors in vivo. Mechanisms of tumor-on-a-chip include microfluidic technology, multicellular co-culture, three-dimensional cell culture, real-time monitoring and analysis, and simulation of tumor-host interactions.

Microfluidic technology forms the foundation of tumor-on-a-chip, utilizing materials such as PDMS, glass, or other biocompatible substances to control fluid flow and distribution through microchannels and chambers [13]. This system enables precise manipulation of culture media, oxygen levels, drugs, and other solutions, simulating dynamic flows of blood and lymphatic fluids in vivo. The technology relies on fluidic dynamics control (via microfluidic pumps and valves for precise regulation of liquid flow rates and volumes) and concentration gradients (creating gradients of drugs, nutrients, and signaling molecules on the chip to simulate complex chemical environments within the body).

4.1.2. Multicellular Co-culture

Tumor-on-a-chip typically involves co-culture of different cell types to simulate the tumor microenvironment. This includes tumor cells (primarily cancer cells for simulating tumor growth and

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invasion), stromal cells (such as fibroblasts for supporting extracellular matrix production and providing structural support), endothelial cells (forming microvascular structures to mimic tumor angiogenesis and provide structural support), and immune cells (such as T cells and macrophages for studying immune evasion and immunotherapy of tumor cells) [14].

4.1.3. Three-dimensional cell culture

This includes extracellular matrix simulation [15] and tissue engineering techniques. Extracellular matrix simulation utilizes hydrogels and other materials to provide structural and mechanical support similar to the ECM in vivo, promoting normal cell morphology and function. Through tissue engineering techniques, three-dimensional models containing tumor cells, stromal cells, and immune cells are constructed to replicate tumor microenvironments and heterogeneity.

4.1.4. Real-Time monitoring and analysis

Tumor-on-a-chip integrate various sensors to monitor cell behavior and microenvironmental changes in real-time. This includes optical sensors and microscopy for observing cell morphology and activities, as well as electrochemical sensors for detecting metabolic products such as glucose and lactate to reflect metabolic activities. Additionally, parameters such as temperature, pH, and oxygen concentration in the microenvironment are analyzed.

4.1.5. Simulation of tumor-host interactions

Through cell-to-cell communication and changes in mechanical environments, tumor-on-a-chip simulate interactions between tumor cells and host cells. Microfluidic channels and cell gaps allow cells to release and receive cytokines, chemical factors, and other signaling molecules, mimicking intercellular communication. By adjusting chip structures and materials, the mechanical environments where cells are located are simulated, such as fluid shear stress and substrate stiffness, which influence cell behavior and drug responses.

4.2. Advantages and disadvantages of tumor-on-a-chip

The tumor-on-a-chip exhibits high physiological and clinical relevance, offering a more realistic simulation of tumor three-dimensional structures and microenvironments, including blood flow, cell interactions, and nutrient exchange. It provides strong controllability, allowing precise control of experimental conditions such as fluid flow rates, drug concentrations, and cell types, resulting in highly reproducible experimental data. It reduces reliance on animal experiments, aligning with ethical and moral requirements. It promotes the development of personalized medicine by utilizing tumor cells from patients for personalized drug screening and treatment optimization, contributing to the advancement of precision medicine.

However, the tumor-on-a-chip faces challenges such as technical complexity, requiring multidisciplinary expertise and sophisticated technical equipment for establishing and operating tumor models. It involves high costs in manufacturing and maintenance, including expensive microfluidic devices, sensors, and cell culture materials. The complexity of data processing, with integrated sensors generating large amounts of real-time data, poses challenges in batch analysis and data handling.

5. Discussion

Regarding the development of mouse tumor models, despite significant progress in the clinical relevance of GEMMs and PDX models, different mouse models still have distinct advantages and disadvantages. Cell line transplantation models (subcutaneous and orthotopic tumor models) and chemical induction models offer control and reproducibility using standardized tumor cell lines or carcinogens, ensuring consistency and repeatability in experiments at lower maintenance costs. However, they poorly simulate the tumor microenvironment compared to the human body, leading to lower clinical relevance.

GEMMs model specific gene mutations and molecular mechanisms of human cancers precisely through genetic manipulation, while PDX models maintain the heterogeneity of patient tumors,

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exhibiting high clinical relevance. Both models require complex technologies, with high costs in establishment and maintenance, and they also lack experimental reproducibility.

In the progression from mouse tumor models to tumor-on-a-chip, the tumor-on-a-chip reduces reliance on experimental animals, meeting ethical requirements for animal experiments. With the industrialization and standardization of tumor chip production, it combines the advantages of both stages of mouse tumor models, namely experimental reproducibility and high clinical relevance. Whether in terms of future scale production costs or model standardization, tumor-on-a-chip undoubtedly represent significant progress compared to mouse tumor models.

However, the future development of tumor-on-a-chip still faces substantial challenges [16]. Firstly, accurately simulating various types of cells, including tumor cells, stromal cells, and immune cells, and their interactions remains challenging. Secondly, maintaining long-term survival and functional stability of cells on chips, especially simulating long-term therapeutic effects of drugs, remains challenging. Lastly, the high upfront design and manufacturing costs of high-precision chips, as well as ongoing maintenance costs, remain high.

Regarding the development of tumor disease models, it is evident that tumor-on-a-chip continually integrate the advantages of both types of mouse models, moving towards standardization, repeatability, and high clinical relevance. The future development in this field will also require interdisciplinary collaboration with experts from other fields, such as microfluidic technology and biomechanics, to collectively advance the continuous development and improvement of tumor-on-a-chip.

6. Conclusion

The development of cancer models, from mouse models to tumor-on-a-chip, highlights the ongoing need to address issues of model construction costs, maintenance costs, experimental reproducibility, and clinical relevance. Despite the significant advancements from mouse tumor models to tumor-on-a-chip, the latter still faces substantial challenges. Future development of tumor-on-a-chip should focus on standardization and regulation, strengthening multidisciplinary collaboration to continuously improve tumor-on-a-chip.

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