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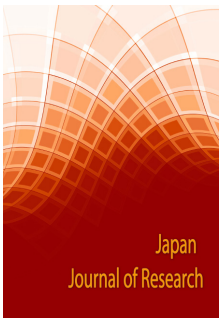

Application And Comparison of Various Mouse Models in Liver Cancer Research

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Abstract

Liver cancer remains a significant global health burden, necessitating robust preclinical models to study its pathophysiology and develop effective therapies. This review explores the utilization and comparative effectiveness of various mouse models in liver cancer research, including subcutaneous tumor models, orthotopic tumor models, spontaneous models, and humanized models. Each model's relevance to mimicking human liver cancer pathophysiology and its implications for therapeutic development are discussed. The strengths and limitations of each model are highlighted, providing insights into their suitability for specific research objectives.

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Introduction

Liver cancer, particularly hepatocellular carcinoma (HCC), is a major global health burden and one of the leading causes of cancer-related deaths worldwide [1,2]. Its high mortality rate is largely due to late-stage diagnosis, limited therapeutic options, and the complex interplay of genetic, environmental, and viral factors that drive its development [3]. Chronic infections with hepatitis B virus (HBV) and hepatitis C virus (HCV), excessive alcohol consumption, non-alcoholic fatty liver disease (NAFLD), and exposure to environmental carcinogens such as aflatoxins are among the key risk factors contributing to the pathogenesis of HCC. Despite significant advancements in diagnostic techniques and therapeutic approaches, the prognosis for HCC patients remains dismal, with a five-year survival rate of less than 20% [4,5]. This underscores the urgent need for a deeper understanding of the disease mechanisms and the development of more effective, targeted therapies.

In the pursuit of unraveling the complexities of liver cancer, reliable animal models have become indispensable tools for studying disease mechanisms, tumor progression, and therapeutic interventions [6]. Among these, mouse models have emerged as a cornerstone of biomedical research due to their genetic similarity to humans, relatively low cost, and ease of genetic and environmental manipulation. Mice can be engineered to mimic specific genetic alterations observed in human HCC, and their immune systems can be modulated to study the role of the tumor microenvironment in cancer progression and treatment response [7,8]. Furthermore, mouse models provide a controlled setting to investigate the efficacy and safety of potential therapies before advancing to human clinical trials, thereby bridging the gap between preclinical research and clinical applications.

Mouse models of liver cancer can be broadly categorized into chemically induced models [9], genetically engineered mouse models (GEMMs) [10,11], xenograft models [12], and patient-derived xenograft (PDX) models [7]. Chemically induced models, such as those using diethylnitrosamine (DEN) or carbon tetrachloride (CCl4), replicate the stepwise progression of liver injury, inflammation, fibrosis, and tumorigenesis, closely mimicking the natural history of HCC in humans. GEMMs, on the other hand, allow for the targeted manipulation of specific genes involved in HCC pathogenesis, such as Myc, Ras, and β-catenin, providing insights into the molecular drivers of tumor initiation and progression. Xenograft models, which involve the transplantation of human cancer cells or tumor fragments into immunodeficient mice, are widely used for preclinical drug testing due to their ability to recapitulate human tumor biology. PDX models, a more advanced form of xenografts, involve the implantation of patient-derived tumor tissues into mice, preserving the heterogeneity and genetic diversity of the original tumor, making them particularly valuable for personalized medicine approaches [13].

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This review aims to provide a comprehensive examination of the various mouse models employed in liver cancer research, focusing on their ability to replicate the heterogeneity and complexity of human HCC. We will evaluate their contributions to advancing our understanding of tumor biology, immune interactions, and therapeutic resistance, as well as their role in the development of novel therapeutic strategies. By highlighting the pivotal role of mouse models in liver cancer research, this review underscores their value in bridging the gap between preclinical discoveries and clinical applications. Ultimately, these models are instrumental in paving the way for more personalized and effective treatment strategies for HCC patients, offering hope for improved outcomes in this devastating disease.

Subcutaneous Tumor Models

Subcutaneous tumor models include PDX models, which utilize human tumor tissues, and syngeneic mouse models, which employ mouse-derived cancer cells in immunocompetent hosts, each offering unique advantages for preclinical cancer research. They are primarily employed for the rapid screening of potential therapeutics. The superficial location of the tumor allows for straightforward monitoring of tumor growth through caliper measurements or imaging techniques such as ultrasound or bioluminescence imaging. This ease of monitoring makes subcutaneous models particularly useful for high-throughput drug screening, where the goal is to quickly identify compounds with antitumor activity [14]. Additionally, these models are often used to study the pharmacokinetics and pharmacodynamics of novel drugs, as well as to evaluate their toxicity profiles. Additionally, they are relatively inexpensive to establish and maintain compared to more complex models, such as orthotopic or genetically engineered mouse models. This makes them accessible for laboratories with limited resources. Most importantly, the subcutaneous environment provides a consistent and controlled setting for tumor growth, leading to high reproducibility across experiments. This is critical for generating reliable and comparable data in preclinical studies [15,16].

Despite their advantages, subcutaneous tumor models have several limitations that must be considered. First, the subcutaneous site does not fully replicate the liver-specific microenvironment, including interactions with hepatocytes, stromal cells, and other immune cells, which can limit the translational relevance of findings, particularly for therapies targeting the tumor microenvironment [17,18]. Secondly, tumors grown subcutaneously rarely metastasize, restricting their utility for studying metastatic processes and therapies aimed at preventing or treating metastasis [19]. Finally, PDX models typically require immunodeficient mice to prevent rejection of human-derived tumor cells, which limits the ability to study immune-mediated therapies or the role of the immune system in tumor progression [20]. These limitations highlight the need for complementary models to address specific research questions in liver cancer [19,21].

Orthotopic Tumor Models

Orthotopic tumor models are a critical tool in liver cancer research due to their ability to closely mimic the natural tumor microenvironment and progression of hepatocellular carcinoma (HCC) [22]. These models involve the implantation of cancer cells or tissues directly into the liver, the organ of origin, thereby preserving the organ-specific interactions between tumor cells and their surrounding microenvironment [23]. Orthotopic models can be broadly categorized into three main types: transplantation models, chemically induced models, and GEMMs. Each of these approaches offers unique advantages and limitations, making them suitable for different research objectives [6].

Transplantation models involve the direct injection of cancer cells or tumor fragments into the liver of recipient mice. These models can be further divided into syngeneic models, which use mouse-derived cancer cells in immunocompetent hosts, and xenograft models, which involve the implantation of human cancer cells or patient-derived tumor tissues into immunodeficient mice. Additionally, chemically induced orthotopic models are established by administering carcinogens such as DEN or carbon CCl4 to induce liver injury, inflammation, fibrosis, and subsequent tumorigenesis [24]. These models recapitulate the stepwise progression of HCC, from chronic liver damage to the development of malignant tumors, making them highly relevant for studying the pathogenesis of HCC and the effects of environmental risk factors. Furthermore, GEMMs are created by introducing specific genetic alterations associated with HCC into the mouse genome [25]. GEMMs are particularly useful for investigating the role of specific signaling pathways and genetic mutations in HCC development [26].

However, orthotopic tumor models have several limitations that must be considered. Firstly, the surgical procedures required for orthotopic implantation are technically demanding and require specialized expertise, which can limit their accessibility for some research teams. Second, tumor growth in orthotopic models is not easily visible, necessitating advanced imaging techniques such as MRI, CT, or bioluminescence imaging for monitoring, which adds to the complexity and cost of these studies. Finally, orthotopic models are generally more expensive and time-consuming to establish and maintain compared to subcutaneous models, making them less suitable for high-throughput screening or large-scale experiments. These challenges highlight the need for careful consideration when selecting an appropriate model for liver cancer research.

Humanized Models

In liver cancer research, developing models that replicate the immune microenvironment based on patient-derived tumors is crucial for precision medicine. Currently, two models can simulate the interaction between patient-derived tumor cells and the immune microenvironment. Specifically, the first is the tumor immune organoid model, which has the advantage of preserving the original tumor's immune microenvironment from the patient. However, the drawback is that immune cells in this in vitro model undergo rapid apoptosis, and the time window for drug sensitivity testing is narrow (approximately 2 weeks), limiting the study to short-term tumor-immune interactions [27-29]. The second approach involves the construction of humanized mice. This can be achieved by isolating CD34+ hematopoietic stem cells from human peripheral blood or umbilical cord blood and transplanting them into immunodeficient mice. Alternatively, fetal liver and thymus tissues from mice can be implanted into immunodeficient mice. The methods of implantation include tail vein injection, bone marrow cavity injection, and intracardiac injection, among others [30,31]. Humanized mouse models are crucial for evaluating human-specific therapeutic responses and understanding the role of the human immune system in liver cancer progression [32]. Additionally, they offer high translational relevance due to the presence of human cells or tissues, making them invaluable for preclinical testing of immunotherapies and targeted therapies [31,33].

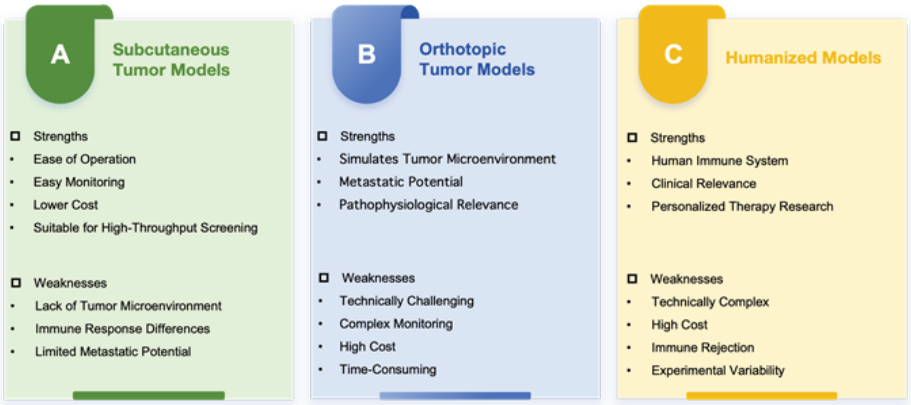

Figure1. Summary of the strengths and weaknesses of three mouse models.

Strengths and Limitations of Mouse Models in HCC Research

Each of these models has unique strengths and limitations. For instance, chemically induced models are advantageous for studying the role of chronic liver injury and inflammation in tumorigenesis, but they may not fully capture the genetic complexity of human HCC. GEMMs offer precise control over genetic alterations but often lack the heterogeneity seen in human tumors. Subcutaneous models are ideal for high-throughput drug screening, while orthotopic models provide more clinically relevant data for studying tumor biology and metastasis. Despite these limitations, mouse models have significantly advanced our understanding of HCC biology, including the roles of oncogenic signaling pathways, tumor-stroma interactions, and immune evasion mechanisms. The choice of model depends on the specific research question, with considerations for cost, technical feasibility, and translational relevance.

Additionally, advancements in genetic engineering, such as CRISPR/Cas9 technology, and the development of more sophisticated humanized models hold promise for improving the predictive power of mouse models in liver cancer research. Furthermore, integrating multi-omics approaches and advanced imaging techniques will enhance our understanding of tumor biology and therapeutic responses. The advent of advanced technologies, such as single-cell RNA sequencing, has further enhanced the utility of mouse models in liver cancer research. These tool enables researchers to dissect the molecular and cellular heterogeneity of tumors and their microenvironment at unprecedented resolution, uncovering novel therapeutic targets and biomarkers. For instance, single-cell RNA sequencing of tumors from GEMMs has identified distinct subpopulations of tumor cells with different metastatic potentials, providing new insights into the mechanisms of tumor dissemination and recurrence.

Conclusion

In conclusion, mouse models have become indispensable tools in liver cancer research, offering valuable insights into the molecular and cellular mechanisms driving tumorigenesis and progression. Their ability to replicate key aspects of human HCC, combined with their versatility and adaptability, makes them ideal for studying disease mechanisms, testing novel therapies, and advancing personalized medicine. As our understanding of liver cancer continues to evolve, mouse models will remain at the forefront of efforts to develop more effective treatments and improve outcomes for patients with this devastating disease. By leveraging the strengths of these models and addressing their limitations, researchers can continue to make significant strides in the fight against liver cancer, bringing us closer to a future where this disease can be effectively prevented, diagnosed, and treated.

Competing interest statement

The authors declare non-competing financial interests.

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