Hepatocellular Carcinoma: Animal Models Available to Characterize Tumor Immunology and Optimize Treatment Development

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Abstract

Hepatocellular carcinoma is the second cause of cancer related-death worldwide with few therapeutic options. In this field, the rate of bench-to-bed translation of drugs is very modest, with 70% of drug attrition suggesting that preclinical evaluation should be urgently improved. Besides, recently immunotherapy showed very promising results and constitutes a real breakthrough in the area of HCC treatment. As these treatments aim to restore anti-tumor immunity, animal models including a functioning immune system are essential to allow a relevant preclinical validation. Macek Jilkova et al. describe, in a clear and forceful review which animal models can optimize this research and what are the pros and cons of each of them.

Introduction

Hepatocellular carcinoma (HCC) is the second cause of cancer-related death worldwide with almost 1 million new cases per year. At the diagnosis, 70% of patients have only access to a palliative treatment [1,2] with few therapeutic options mostly represented by tyrosine kinase inhibitors such as sorafenib [3] and lenvatinib [4] in first line; regorafenib [5] and cabozantinib [6] in second line. HCC occurs on a cirrhotic liver in more than 90% of cases and liver is a singular organ from an immunological point of view. Cirrhosis modulates liver immune landscape through chronic alterations such as inflammation and fibrosis and these immune changes may induce aberrant immunotolerance through the activation of multiple pathways involving major immune functions such as antigen presentation or lymphocytes' exhaustion. These modifications lead to failure to immunosurveillance systems and allow tumor initiation and progression [7]. For that reason, HCC seems to be a good candidate to immunostimulatory therapies aiming to restore anticancer immunity. These therapies are currently strongly studied

in this pathology with the advent of monoclonal antibodies directed against immune checkpoints, especially against PD-1/PD-L1 pathway. Two new combination therapies particularly stand out: atezolizumab (anti-PD-L1) - bevacizumab (anti-angiogenic) which is becoming the new standard in first line [8] and durvalumab (anti-PD-L1) - tremelimumab (anti-CTLA4) [9]. Nonetheless, a large proportion of patients do not respond to these treatments and complex physiopathological mechanisms involved in HCC oncogenesis, as well as resistance pathways activated during these immunotherapies are still poorly understood.

Early Drug Development Phases in HCC: A Major Challenge

New therapies are urgently needed, and the use of combinations targeting multiple pathways seems to be the most promising approach. However, due to cumulative toxicities, preclinical phases are essential before testing these innovative therapeutic schemes in humans. To this day, the rate of bench-to-bed translation of candidate anticancer drugs is very modest, with the failure in clinical

Animal models in HCC	Advantages	Disadvantages
Chemically induced [16-19]	Fully functional immune system Respect of inflammation-fibrosis-cancer sequence	Delay of tumor induction High mutational burden differing from most human situations
Genetically engineered [20]	Specific study of an oncogene Fully functional immune system	Overrepresentation of mutated oncogenes/tumor suppressors Lack of inflammation-fibrosis-cancer sequence
Syngeneic [21-23]	Fully functional immune system Metastasis formation	Limited similarity to human HCC Lack of inflammation-fibrosis-cancer sequence
Xenograft [13,14]	Low cost and rapid Easy to measure tumor progression	Lack of inflammation-fibrosis-cancer sequence Lack of functioning immune system
Humanized [24-27]	Functional immune system with presence of specific actors of human cancer immunology Relevant genetic landscape Particularly relevant to study immunotherapy	High cost, high level of expertise and high-tech facility Lack of inflammation-fibrosis-cancer sequence

Table 1: Summary of different animal models available to study anticancer drugs efficacy and consequences.

phases of approximately 70% of drugs initially considered as hopeful based on preclinical data [10,11]. Consequently, preclinical drug studies need to be improved to optimize drug selection and better study their efficacy and safety in a relevant manner. Indeed, several issues are usually encountered in classical drug development using inadequate animal models such as xenograft. These models are useful in large-scale drug testing because they are cheap, and a rapid- and easy-to-assess tumor growth, but they present many limits [12]. Mainly, xenograft models do not reproduce tumor heterogeneity. In fact, they present an irrelevant tumor microenvironment due to a non-functional immune system and the absence of organ chronic alterations preceding cancer such as cirrhosis, in the case of HCC [13,14]. The absence of underlying cirrhosis in the vast majority of animal models used in preclinical studies related of HCC, is probably one of the main causes of the high rate of drug attrition, and this concerns either cytotoxic chemotherapies, target therapies, or immunotherapies. Finally, recent studies suggest that many drugs tested in humans are probably targeting superfluous pathways, due to an insufficient knowledge of true driver genes involved in cancers [15]. In all, these elements suggest that specific animal models are needed to help research in the field and optimize anticancer drug development.

Animal Models Available to Better Characterize HCC Immunology and Optimize Drug Selection

Macek Jilkova et al. proposed a well-documented and illustrated review detailing the most relevant animal models available to study HCC immunology and immunotherapies [28]. Models include generation of tumors by chemical agents, the use of genetically engineered mice (GEM), or utilization of syngeneic and humanized animals. Each model presents advantages and limits summarized in Table 1; however, it is important to mention that every model remains artificial for various reasons.

For example, chemically induced models using carcinogens such as diethylnitrosamine (DEN) are interesting as they reproduce the physiopathological sequence of chronic inflammation-fibrosis-cirrhosis preceding cancer [16-19]. The DEN model also is relevant because it keeps a functional immune system. Nonetheless, DEN is a strong

carcinogen which induces multiple DNA alterations that do not correspond precisely to the mutational landscape of the majority of human HCCs.

Models such as GEM allow for a better study of the impact of a specific genetic alteration and are developed in mice with a functional immune environment as well. These models are particularly interesting to study the driver genes and the main pathways involved in tumor initiation and progression. For example, the transgenic AlbLTαβ model with aberrant expression of the cytokine lymphotoxin helped to better characterize inflammation-induced hepatocarcinogenesis [20]. Nevertheless, GEM suffer from a low and stereotypic mutational burden, and they usually do not present the inflammation-fibrosis-cirrhosis sequence. Also, even though their development duration can be shortened through accumulation of several mutations, or the use of new techniques such as CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9, these models stay time-consuming, expensive, and require large infrastructures.

Other models that are particularly interesting to reproduce the complexity of the tumor microenvironment in mice with full functioning immune system are the syngeneic models. They consist of the injection in immunocompetent mice, of tumor cells derived from the same species, directly in the organ which is concerned by the cancer being studied. They offer the possibility to study all key players of the immune system and tumor microenvironment, including tissue architectural considerations such as the vasculature, the stroma, and the surrounding lymphatic system [21]. Another advantage is their potential to develop metastases, which is particularly relevant to study concepts such as tumor invasion or metastatic homing, and their relation with tumor immune microenvironment [22]. However, one of the most important limits of these models resides in their mutational landscape that differs from humans with different driver genes involved in the tumorigenesis process. Moreover, as the immune system is still functional in these animals, the implication of aberrant immunotolerance in tumorigenesis cannot be observed and may lead to spontaneous tumor regression, which increases the distance with the human situation [23].

Thus, the development of models better mimicking HCC pathogenesis in human with realistic tumor-immune system interactions represents a major challenge to improve drug study. Humanized animal models constitute a very valuable tool to study these elements. Indeed, they are based on the engraftment of human immune cells such as peripheral blood lymphocytes or CD34+ hematopoietic stem cells, in severe combined immunodeficient rodents [24-26], followed by tumor xenografts such as patient derived xenografts [27]. Humanized models are very attractive to improve anticancer drug development such as immunotherapy as they reproduce the complexity and specificity of humans HCC and their interactions with immune system. Humanized mice, however are still poorly used in HCC field, mostly due to their high cost and the high level of technicity that they require.

Conclusion

Oncology research is constantly evolving with many new candidate therapies which are more and more focused on specific pathways involved in multiple mechanisms such as tumor progression, angiogenesis or tumor-immune system interactions. Besides, the high level of drug attrition from the bench to the early clinical phases, strongly suggest that the use of old models such as basic xenografts models is not appropriate. Thus, the use of innovative models that respect crucial principles present in HCC such as a strong tumor heterogeneity, the presence of an underlying cirrhosis and a complex and singular immune system, is essential and should be systematic to develop and assess future anticancer drugs in a relevant manner. The review proposed by Macek Jilkova et al. offers a very clear overview of preclinical models available to study liver immunology and its implications in HCC, by demonstrating the pros and cons of each animal model.

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