Translational Oncology
www.transonc.com

Volume 12 Number 7 July 2019 pp. 987–995 987

Cancer Immunotherapies and Humanized Mouse Drug Testing Platforms[1]

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Abstract

Cancer immunotherapy is a type of treatment that restores and stimulates human immune system to inhibit cancer growth or eradicate cancer. It serves as one of the latest systemic therapies, which has been approved to treat different types of cancer in patients. Nevertheless, the clinical response rate is unsatisfactory and the response observed is mostly a partial response in patients. Despite the continuous improvement and identification of novel cancer immunotherapy, there is a pressing need to establish a robust platform to evaluate the efficacy and safety of pre-clinical drugs, simulate the interaction between patients' tumor and immune system, and predict patients' responses to the treatment. In this review, we summarize the pros and cons of existing immuno-oncology assay platforms, especially the humanized mouse models for the screening of cancer immunotherapy drugs. In addition, various emerging trends and progress of utilizing humanized mouse models as the screening tool are discussed. Of note, humanized mouse models can also be used for further development of personalized precision medicines to treat cancer. Collectively, these highlight the significance of humanized mouse models as the important platform for the screening of next generation cancer immunotherapy *in vivo*.

Translational Oncology (2019) 12, 987–995

Introduction

Over the past decade, various types of cancer therapy (such as surgery, chemotherapy, radiotherapy, bone marrow transplantation, hormone therapy, targeted drug therapy and cryoablation treatment) have been applied to treat cancer, albeit these therapies are often associated with different side effects and limited responses in controlling tumor growth as reported in many clinical cases [1,2]. Moreover, patients' immune system, which plays a crucial role in the progression of cancer, is not taken into consideration in these treatments. In recent years, cancer immunotherapy has been extensively studied in oncology and oncopharmacology research, and the recent success of immune checkpoint blockades (ICBs) and chimeric antigen receptor (CAR) T-cell therapy in the clinic also reveals the significance of cancer immunotherapy [3]. Currently, more than 3000 kinds of cancer immunotherapy, including ICBs, cancer vaccines, oncolytic

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[1] Acknowledgments: This study was supported by MOH Industry Alignment Fund Cat 2 (MOHIAFCAT 2001), Open fund-large collaborative grant (NMRC/OFLCG/003/2018) and by the Eradication of HBV TCR Program: NMRC/TCR/014- NUHS/2015 and NMRC/TCR/015-NCC/2016 National Medical Research Council Singapore. Qingfeng Chen is also supported by the National Research Foundation Fellowship Singapore NRF-NRFF2017-03.
Received 14 April 2019; Revised 26 April 2019; Accepted 28 April 2019

1936-5233/19
https://doi.org/10.1016/j.tranon.2019.04.020

virus, cytokine therapy, cell therapy (CAR-T, TCR-T and CAR-NK), and the combination of immunotherapy that targets multiple immune components are under rapid development, and some of the treatments are further assessed in clinical trials [4]. In view of the drastic increase in the demand for cancer immunotherapy, it is expected that the market of cancer immunotherapy will reach more than US$100 billion by 2024 [5]. Although there are several promising drugs in the pipeline, it is still an unprecedented challenge to pharmaceutical industry due to the extremely high cost and failure rate in clinical trials. Therefore, a robust and reliable platform is essential for screening single or combinatorial cancer immunotherapy drugs, which can minimize the discrepancy between the findings from experiments and clinical trials, and predict patients' response to the cancer treatment.

In 1777, the first attempt of cancer vaccine was performed by injecting malignant tissue [6]. Ever since then, a huge number of theories in cancer immunotherapy have been introduced, which are accompanied by the development of numerous immuno-oncology assays to validate the novel concepts [6]. Nevertheless, due to different limitations, including ethical and economic issues, as well as the stringent patient selection criteria in clinical trials, most of these immuno-oncology assays have to be performed in pre-clinical stage, which can be categorized into *in vitro* and *in vivo* platforms in general [7–13].

For *in vitro* studies, two-dimensional (2D) or three-dimensional (3D) co-culture systems, using either cells or organoids are well-established, which allow us to evaluate large number of drugs via high throughput screening, and these systems are relatively less costly and less time-consuming when compared to animal studies [7–10,14]. Regardless of the advantages of the *in vitro* systems, a lack of tumor-specific 3D complex structure hampers the studies of crosstalk among cancer immunotherapy drugs, tumor cells, non-tumor cells and the microenvironment, such as the presence of abnormal vascularization and drug barrier [15]. To overcome the limitation of the *in vitro* systems, different animal models, including Caenorhabditis elegans, drosophila, dog, monkey and mouse model have been established, and mouse cancer model is the most commonly used platform for testing cancer immunotherapy among these models [16–21]. However, the interaction between human immune response and human tumor cannot be investigated using wildtype or immunodeficient mouse models, and the clinical relevance for screening the human-specific cancer immunotherapy drugs is hindered [20]. Recently, several reports have demonstrated the crosstalk between human immune response and human tumor using humanized mouse cancer models, which provide a promising tool in pre-clinical cancer immunotherapy research [12,13,22]. Therefore, further characterization and continuous improvement of the humanized mouse cancer models, especially the development of autologous human immune system and tumor in humanized mice, are critical for the evaluation of novel cancer immunotherapy agents and personalized precision medicines.

In this review, the existing and ongoing development of cancer immunotherapies will be briefly summarized. Moreover, the recent advances in immuno-oncology assays will be reviewed, and we will focus on the applications of humanized mouse cancer models for illustration. Of equal importance, we will discuss the future perspectives on the development of humanized mouse cancer models, and utilize the unique model for the evaluation of next generation cancer immunotherapy.

Cancer Immunotherapy

Immune Checkpoint Blockades

ICBs are a new class of cancer immunotherapy drugs that are designed to increase anti-cancer effects in patients via suppressing multiple immune checkpoints, particularly in cytotoxic T lymphocytes. Apart from the former cell type, the immune checkpoints are also expressed in different immune cell types, including B cells, natural killer cells (NKs), monocytes, tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs) and dendritic cells (DCs). The immune checkpoints primarily exhibit immunosuppressive and effector functions, as well as reducing tissue damage due to uncontrolled T-cell responses [23,24]. Nonetheless, increasing evidences have revealed that the expression levels of various immune checkpoints, such as CTLA-4, PD-1, TIM-3, BTLA and LAG-3 were dysregulated in the tumor-infiltrated T cells [25]. As a result, the T cells were exhausted, and the anti-cancer functions of the immune system were weakened. ICBs could remove these inhibitory signals, restore the T cells from their exhausted status and recover their cytotoxicity on tumor cells [26]. In 2011, Yervoy (or ipilimumab), developed by Bristol-Myers Squibb, was the first ICB to be FDA-approved for treating metastatic melanoma. To date, more than 110 ICB-related drugs have been developed for clinical trials, or FDA-approved to treat cancer patients [27].

Adoptive Cell Therapy

Adoptive cell therapy (ACT) is an immune cell-mediated immunotherapy that destructs tumor cells. In general, various immune cell subsets are isolated from patients or donors, genetically modified and expanded. Subsequently, these cells are transferred back to the patients to elicit the anti-cancer responses [28,29]. The initial study of the ACT was described in 1988, using the adoptive transfer of primary tumor-infiltrating lymphocytes (TILs) to treat patients with metastatic melanoma [30,31]. Over the past decades, several strategies have been developed and more diverse immune cell subsets, such as non-specific lymphokine-activated killer (LAK) cells, adoptive T cells, chimeric antigen receptor (CAR)-T, T cell receptor (TCR)-engineered T cells and CAR-NK cells are involved in the ACT [32–34]. Despite the promising therapeutic values of the ACT, the anti-tumor effects of the LAK cells and TILs are non-specific. In addition, the major obstacles of the ACT include limited expansion capacity and drug targeting efficiency of the effector cells. To overcome these barriers, TCR-engineered T cells, CAR-T and CAR-NK cells are developed, which target specific antigens on cancer cells [35]. TCR-engineered T cells are generated via transfecting cloned antigen-specific TCR genes into the T cells isolated from cancer patients, using either lentivirus or retrovirus, to eradicate specific tumor cells. Due to the expression of antigen-specific genes, TCR-T cells showed optimistic results in sarcoma, metastatic melanoma, lymphoma and leukemia patients [36–41]. Concurrently, CAR-T cells are developed to overcome the limitations raised by MHC restriction in the TCR-based cancer immunotherapy. CAR-T cells compose of an antigen-binding single-chain variable fragment (scFv) domain, a signal transduction domain and a transmembrane domain, which allow the CAR-T cells to recognize tumor cells in MHC-unrestricted manner [42,43]. In addition to the advantages of CAR-T cells, CAR-NK cells have also attracted great interest in clinical settings due to their conceivable cytotoxicity against tumor cells, and

the infused CAR-NK cells will not induce graft-versus-host disease in cancer patients [34].

Cancer Vaccines

Cancer vaccines refer to the administration of tumor-associated antigens to restore and stimulate specific anti-cancer immune responses [44]. Based on their application, the vaccines can be divided into preventive or therapeutic vaccines [45], which can be further grouped into virus, peptide, DNA or DC vaccines, depending on the sources of the antigens [46–48]. Until now, FDA has approved several cancer vaccines for clinical uses such as Provenge, hepatitis B virus (HBV) vaccines and human papilloma virus vaccines. [46,49,50]. Meanwhile, many potential cancer vaccines are currently under investigation in clinical trials, including DNA-containing liposomes and nanoparticles (DNA vaccines), and gp100 peptide (peptide vaccines), which may lead to significant clinical benefits in the future [51–53].

Oncolytic Virus

Oncolytic virus immunotherapy represents a class of immunotherapy that utilizes genetically engineered or naturally occurring viruses. These viruses preferentially lyse cancer cells and elicit adaptive anti-tumor immunity by activating DCs with damage-associated molecular patterns and tumor-associated antigens, while causing minimal side effects to normal cells [54]. T-VEC is the most frequently used oncolytic virus for melanoma treatment in patients, which was approved by US FDA in 2015, and approved by Europe FDA in 2016 [55]. Recently, a growing number of oncolytic viruses have been investigated in clinical trials, and some of the viruses are pending for FDA approval [56–63].

Cytokine Therapy

Cytokine therapy can result in tumor destruction through different mechanisms. On one hand, the cytokines act on the tumor cells directly to inhibit their proliferation by inducing apoptosis, suppressing angiogenesis and modulation of their differentiation. On the other hand, the anti-cancer immune response can be triggered by the administration or blockade of specific cytokines which interfere with the corresponding signaling pathways [64,65]. Although cytokine therapy alone showed favorable anti-cancer responses in some studies, a combination of cytokine therapy with other immunotherapy, such as ICBs, adoptive cell therapy and cancer vaccines may plausibly improve the efficacy of the anti-cancer effects [66,67].

Targeting Immunosuppressive Tumor Microenvironment

Tumor microenvironment plays a crucial role in cancer growth, development and metastasis. Depleting various immunosuppressive cellular elements from the tumor microenvironment, such as tumor-specific regulatory T cells, type 2 helper T cells, regulatory B cells, innate lymphoid cells, type 2 granulocytes (Neu2), DC of type 2, TAM and MDSC were reported to restore and re-activate the anti-cancer immune responses [68–70].

Combinational Immunotherapy

Earlier clinical reports have demonstrated the capability of single cancer immunotherapy agent to treat patients, whereas a subgroup of patients with advanced stage of cancers may not respond well to the single-agent treatment. Hence, there is an increasing trend to combine different immunotherapy drugs, or treatments (chemotherapy and radiotherapy) to enhance the anti-tumor efficacy, through the possible synergistic effects of these treatments. Currently, different ICBs, such as anti-PD-1 and anti-CTLA4 antibodies were combined and administered to cancer patients. The results from these combinational therapies are encouraging and more combination regimens warrant further studies, which may improve the overall outcome of patients with multiple types of cancers [71,72].

Immuno-oncology Assay Platforms

Despite a vast number of drug candidates for cancer immunotherapy, it is challenging to identify the most efficacious drug and optimal dose to treat patients [73]. Moreover, the concept of personalized precision cancer treatment imposes another obstacle for the treatment option. Due to the heterogeneity of individual's tumor, tumor microenvironment and the immune system, there is a considerable variation in individual's responses, including the anti-cancer effects and the side effects towards the same treatment [74]. Therefore, continuous development and improvement of immuno-oncology assay methods are essential to expedite evaluation of single or combinational immunotherapy in cancer research, or predict the clinical outcome of an individual cancer patient.

In Vitro Platforms

Majority of cancer immunotherapy agents were first characterized and evaluated *in vitro* using 2D and 3D models [10]. Human cancer cell lines, cancer cells-derived organoids, primary tumor cells or patient-derived tumor organoids are either cultured alone, or co-cultured with human peripheral blood mononuclear cells (PBMCs), TILs, CAR-T, TCR-T or CAR-NK cells for screening the efficacy of the cancer immunotherapy (Figure. 1). For the 2D screening system, specific cancer cells or organoids are co-cultured with the immune cells on flat dishes [75,76]. Subsequently, cancer immunotherapy agents are added to examine the phenotypic modulation of cells and the underlying molecular mechanisms, through various immune cell assays (for T cells, NK cells, macrophages, DCs and neutrophils) and tumor cell assays. However, the 2D system represents an artificial and simplified cultural environment, while the 3D system is capable of reflecting the complexity of tumors in patients, since the tumors also consist of immune cells, stromal cells, epithelial cells and endothelial cells [77]. The 3D culture system can be classified as scaffold system and scaffold-free system. The former system supports cell growth, proliferation and survival through a specific extracellular matrix (ECM), such as hydrogel. The essential nutrients and growth factors are supplemented to the cells through some small pores in the ECM, which is similar to their primary microenvironment *in vivo* [78]. In contrast, 3D scaffold-free system generates spheroids and cell aggregates by forced-floating, hanging drop or agitation-based technology in the absence of ECM, which provides a better physiological model when compared to the scaffold system.

In Vivo Models

The aforementioned *in vitro* platforms allow us to promptly evaluate the efficacy of cancer immunotherapy drugs. However, the *in vitro* models barely reproduce the complexity of tumor microenvironment, which consists of human tumor cells, immune cells and stromal cells. Hence, there is an urgent need to develop sophisticated *in vivo* models that can mimic the tumor microenvironment in patients for the assessment of novel cancer immunotherapy agents (Figure. 1).

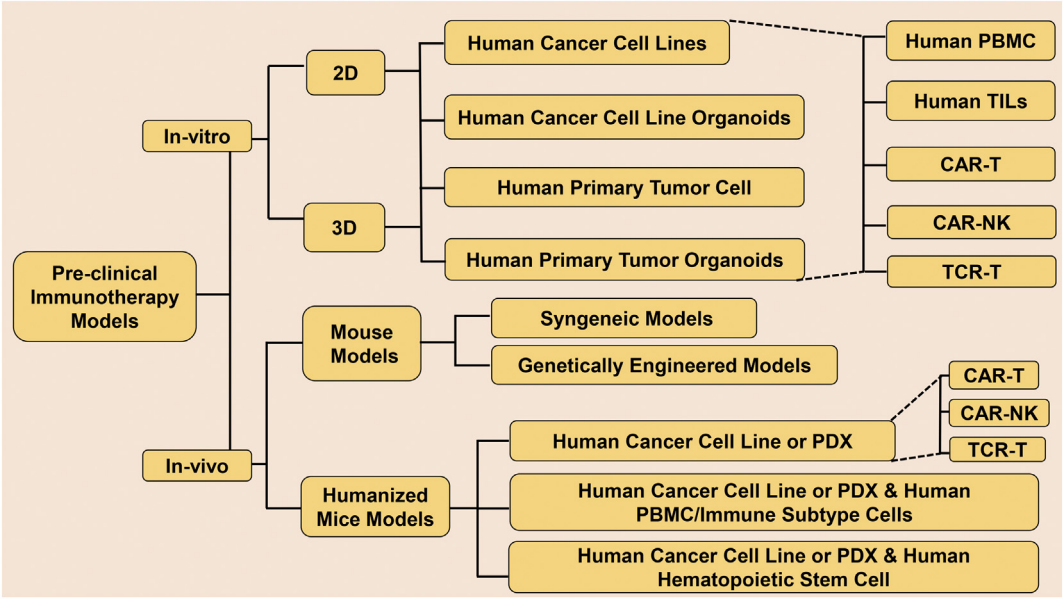

Figure. 1. Key features of *in vitro* and *in vivo* immuno-oncology assays

Syngeneic and Genetically-Engineered Mouse Tumor Models

Over decades, syngeneic mouse tumor models are the major *in vivo* model for tumor immunology and immunotherapy studies, where these models are established by either subcutaneous or intravenous injection of mouse tumor cell lines into inbred mouse strains (immunocompetent mice) [20,79,80]. As a result, these models can be used for studying the efficacy of the anti-tumor immune responses, without the adoptive transfer of immune cells into the mice prior to the studies. The advantages of these models are that they are easy to be handled, can be rapidly and reproducibly expanded in large numbers, and produce relatively consistent results for the evaluation of various factors that influence the efficacy of immunotherapy [79,80].

Generation of genetically-engineered mouse tumor models require either tissue-specific promoters to drive the expression or activation of oncogenes (such as Kras) or recombinase enzymes (including Tet-on/off, tamoxifen-inducible Cre recombinase and CRISPR-Cas9 technology) that remove tumor suppressor genes (such as Tp53), and these models have been widely used to evaluate targeted therapy or other immunotherapeutic modalities [20,81]. Yet, a lack of genomic heterogeneity of the mouse tumors, and the discrepancy of tumor microenvironment between mouse and human are the major caveats of these models. Remarkably, significant differences exist between the immune system in mouse and human, which may possibly reduce the clinical relevance of the syngeneic and genetically

Table 1. Current Humanized Mouse Tumor Models for Human Cancer Immunotherapy

Type of models		Main advantages	Main disadvantages	Applications in immunotherapy
Humanized Oncology Models	Human Tumor Cell Lines engrafted into immunodeficient mice	Easy to handle and can be rapidly and reproducibly expanded in large numbers. Lower variants	Lack of human immune tumor microenvironment. Lack of human tumor heterogeneity.	Adoptive cell therapy Oncolytic virus Cytokine therapy
	Human PDX Tumor engrafted into immunodeficient mice	Patient *tumor h*eterogeneity Similar responses as patients to certain anti-cancer drugs.	Lack of human immune tumor microenvironment.	Adoptive cell therapy Oncolytic virus Cytokine therapy
Humanized Immune-Oncology Models	PBMCs & Tumor Cell Lines or PDX Tumor	Human immune tumor microenvironment Patient *tumor* heterogeneity Same immune system from cancer patients	Graft-versus host reaction (GVHR) and short survival time.	Immune checkpoint blockades Cancer vaccines Cytokine therapy Combinational immunotherapy
	HSCs & Tumor Cell Lines or PDX Tumor	Human immune tumor microenvironment Patient *tumor h*eterogeneity Without risk of GVHR	High cost and limited number of HSCs Different immune system from cancer patients	Immune checkpoint blockades Cancer vaccines Cytokine therapy Combinational immunotherapy

engineered mouse tumor models in the cancer immunotherapy studies [82].

Humanized Mouse Tumor Models

Humanized mouse tumor models can be divided into humanized oncology models and humanized immune-oncology models (Table 1).

Humanized Oncology Models

The humanized oncology models are established by the injection or engraftment of human cancer cell lines (from human tumor or oncogene-generated) or human PDX tumor into immunodeficient mice, including but not limiting to athymic nude mice, severe combined immunodeficiency (SCID) mice, non-obese diabetic (NOD) mice and NOD *scid* gamma (NSG) mice [20,83,84]. Recently, the models have been used to screen the immunotherapy agents against cancers, such as monoclonal or bispecific antibody therapies, CAR-T cells, oncolytic virus and cytokine therapies [84–87]. In human CCR9+ T cell acute lymphoblastic leukemia (ALL) immunodeficient mouse model, 92R monoclonal antibody strongly inhibits tumor growth via binding to the CCR9 N-terminal domain [88]. In another study, human NPM1 gene in acute myeloid leukemia (AML) cells was genetically modified by lentiviruses and the mutant cells (NPM1c+) were engrafted into immunodeficient mice for evaluating the efficacy of CD3 and CD123 bispecific antibody conjugates [89]. Previous studies also demonstrated the anti-HCC effect of anti-GPC3 CAR-T cells therapy in immunodeficient mouse HCC-PDX model [90], and the efficacy of anti-CD19 CAR-T cells therapy against B-cell lymphoma in immunodeficient mice [91]. Similarly, anti-HER2 CAR-T cells therapy could be used to treat ICBs-resistance melanoma in mice [92]. Apart from CAR-T cells therapy, it was reported that the delivery of oncolytic virus, using modified neural stem cells was more efficient and able to extend the survival of mice in mouse glioma-PDX model (GBM43 and GBM) [93]. The interactions of multiple kinds of oncolytic vaccinia virus with tumor are also analyzed in A549-bearing immunodeficient mice [94]. It was also revealed that combination therapy using temozolomide (chemotherapy drug) and ibudliast (macrophage migration inhibitory factor inhibitor) prolonged the survival of mice in glioblastoma-PDX model [95]. However, the major drawback is that ICBs or cancer vaccines cannot be evaluated in these models due to the absence of human immune microenvironment. Therefore, there is a growing interest in developing human immune-oncology models for the studies of cancer immunotherapy.

Humanized Immune-Oncology Models

Humanized immune-oncology models are generated by the engraftment of human cancer cell lines or human PDX tumors into the immunodeficient mice bearing HLA-matched human immune system. The human immune system of these models could be generated by the transplantation of human PBMCs or hematopoietic stem cells (HSCs).

Human PBMCs Models

To reconstitute the human immune system, human PBMCs or mature immune cell subsets are injected into the irradiated NOG or NSG mice by intravenous injection. Human T cells, B cells, NK cells and DCs can be detected in the circulation, and monoclonal antibody, cytokine therapy (IL-2), ICBs and DC-based vaccine have been successfully evaluated in these models [22,96,97]. In PBMC-humanized mouse model (PBMCs from healthy donors), the administration of human monoclonal antibody anti-CAIX, combined with IL-2 could inhibit the progression of renal cell carcinoma via inducing human NK and T cells responses [98]. Anti-tumor activity of nivolumab, atezolizumab, pembrolizumab and urelumab was also observed in the PBMC-humanized mouse model [22,99,100]. Utilizing autologous PBMCs and glioma-PDX, personalized immune-oncology models were established and could be used for evaluating personalized ICBs [101]. Multiple DC vaccine formulations were compared in PBMC-humanized mouse model and assessed for MART-1-specific immune responses and suppressive functions on melanoma [102]. Nevertheless, the development of graft-versus host reaction (GVHR) and poor survival of the mice confines the use of these models to evaluate the effectiveness of cancer immunotherapy [103].

Human HSCs Models

The barriers of GVHR can be overcome by the establishment of human immune system using human $CD34^+$ HSCs. The stem cells can adapt mouse environment under mouse MHC education and develop into a well-reconstituted human immune system without the risk of GVHR in immunodeficient mice. These mouse models have been used to generate more stable humanized immune-oncology models successfully [12,13]. In the FL (fetal liver)-HSC-generated humanized mice, human helper T cells, cytotoxic T cells, B cells, monocytes, NK cells and DCs were detected in the circulation [13]. The number and proportion of cytotoxic T cells and NK cells decreased during HCC-PDX progression [13]. Intriguingly, TILs isolated from the tumors displayed tumor-educated phenotypes, including increased immune checkpoints expression and an impaired cytokines and cytotoxic proteins production. The presence of more type 2 monocytes (M2) and multiple MDSCs could also mimic the immune responses in patients [13]. In the same study, the side effects of ICBs were demonstrated, which is in line with some of the clinical studies [13]. Alternatively, in the CB (Cord Blood)-HSC-generated humanized mouse model, nivolumab inhibited the growth of MDA-MB-231 cells and CRC172 cells by enhancing anti-tumor T cell response, increasing GrB^+ or IFN-γ^+ $CD8^+$ cells in tumors and reducing frequency of HLA-DR^{low} myeloid cells *in vivo* [104]. Meanwhile, combination of HDAC inhibitors OKI-179 and nivolumab further inhibited tumor growth when compared to the administration of nivolumab alone, which also indicated that HDAC inhibitors could improve anti-tumor immune responses *in vivo* [104]. To date, a growing number of reports have shown that human PDX tumors were regressed in the humanized mice following anti-PD1 and anti-CTLA4 therapies [12,13,22], suggesting that the humanized immune-oncology models may serve as the emerging platform for the study of cancer immunotherapy or combinational immunotherapy.

Future Directions of Studying and Developing Humanized Mouse Immune-Oncology Models

Future Applications of Humanized Mouse Immune-Oncology Models

The current humanized mouse cancer models are frequently used to examine the efficacy and the safety of ICBs. Beside T cells, multiple immune cell subtypes, such as monocytes and NK cells also showed the tumor-educated phenotypes in tumor microenvironment. These phenotypes may contribute to tumor progression and may plausibly be the drug targets in the future [13]. Moreover, these models can be

used to explore the potential combinations of immunotherapy to treat patients and predict the outcomes.

Next-Generation Humanized Mouse Immune-Oncology Models

The main drawback of the current human HSC-derived immune-oncology models is that the human tumor and immune system are not usually from the same donors, thus they are only HLA partially matched. We anticipate that the future directions for immune-oncology study and drug testing is to develop HLA fully matched and personalized humanized mouse models. The possible option is to match PDX tumors with the same patient derived HSCs. This requires the resources of human HSCs derived from induced pluripotent stem cells (iPSC), peripheral blood or bone marrow. Besides immune cells, iPSCs have the possibility to further differentiate into vascular cells and fibroblasts, which may increase the similarity of the patients' tumor microenvironment in the humanized mice [105–108]. However, a major challenge of iPSC-derived HSCs is their low *in vivo* repopulating ability which results in the difficulty of forming functional immune system in immunodeficient mice [109,110]. In addition, it is imperative to develop fully HLA-matched humanized mouse immune-oncology models using patients' HSCs from their PBMCs or bone marrow cells [111,112]. These models may reflect patients' immune responses more accurately when compared to the current partially HLA-matched models. Nevertheless, human bone marrow cells have limited capacity to develop into immune cells in humanized mice, which may lead to insufficient number of fully HLA-matched humanized mice for cancer immunotherapy studies.

In addition, further improvement of mouse environment is warranted to optimize human immune cell development and functionality in humanized mice. For instance, many cytokines and growth factors do not cross react between mouse and human, which lead to the sub-optimal development of specific human immune cell types in humanized mice, such as human NK cells and macrophages due to the lack of human interleukin (IL)-15 and macrophage colony-stimulating factor (M-CSF), respectively [113]. Furthermore, the residual mouse immune components, which mainly consist of macrophages and granulocytes may also interfere the responses. Efforts have been made to improve the human immune elements in the humanized mice, such as transgenically expression of different human growth factors and cytokines [112,114], hydrodynamic tail vein injection of plasmid DNA encoding human cytokines [113], lentivirus or adeno-associated virus delivery of cytokines [115–117] and replacing the host genes with human counterparts [118–120]. Using genetically engineering technology, NSG-SGM3 mice (genetically engineered to produce IL-3, granulocyte/macrophage colony-stimulating factor (GM-CSF) and Steel factor (SF)), NSGW41 mice (KitW-41J allele), MISTRG (co-expressed M-CSF, IL-3, GM-CSF and thrombopoietin) and NSG-IL-15 (high expression of human IL-15) were established for improving the development and differentiation of HSCs, NKs and monocytes/macrophages [121,122]. Additionally, overexpression of human IL-2, IL-7, IL-15 and SF in humanized mice by hydrodynamic injection of vector, lentivirus or adeno-associated virus also contributes to the development of human HSCs [96,113,118]. Yet, there is still room for further characterization of these methods and exploration of new factors can help to achieve fully functional human immune system in humanized mice, which is valuable for the subsequent validation of single-agent or combinational immunotherapies in the humanized mouse immune-oncology models.

Taken together, humanized mouse cancer models are becoming more and more commonly used to evaluate the efficacy and safety of cancer immunotherapy *in vivo*. Timely improvement of these models is essential as more novel immunotherapy drugs or combinations of immunotherapy have been explored. Besides, next generation humanized mouse immune-oncology models, which closely simulate the interaction between the tumor and immune system in cancer patients have to be generated, so as to increase the clinical relevance of immunotherapy and enhance the development of personalized precision medicines.

Conflict of Interest Statement

The authors declare that we have no conflict of interest.

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