Humanized Rodent Models for Cancer Research

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As one of the most popular laboratory animal models, rodents have been playing crucial roles in mechanistic investigations of oncogenesis as well as anticancer drug or regimen discoveries. However, rodent tumors show different or no responses to therapies against human cancers, and thus, in recent years, increased attention has been given to mouse models with xenografted or spontaneous human cancer cells. By combining with the human immune system (HIS) mice, these models have become more sophisticated and robust, enabling *in vivo* exploration of human cancer immunology and immunotherapy. In this review, we summarize the pros and cons of these humanized mouse models, with a focus on their potential as an *in vivo* platform for human cancer research. We also discuss the strategies for further improving these models.

Keywords: humanized mice, rodent, immunology, cancer, immunotherapy

INTRODUCTION

The high morbidity and mortality rates associated with various cancers across the globe clearly indicate that cancer-related research is one of the fastest-growing fields in the world (1). Although great progress has been made in understanding the underlying mechanisms of cancer and the discovery of anticancer drugs in the past few decades, efficient clinical translation of these technologies remains very limited (2). One of the main reasons for this is that most of these studies rely on rodent models, which have a number of important physiological differences from humans. Thus, rodent cancer models cannot accurately simulate the physiology of cancer patients, which necessitates the development of novel animal models that are better equipped to precisely and comprehensively represent the complex feature of human cancers allowing for improved basic and translational investigations (3).

Animals bearing tumors of human origin were first developed using T-cell-deficient nude mice inoculated with human cancer cell lines [now known as cell-derived xenograft (CDX) models] and these rodent models became a popular *in vivo* platform to study human oncogenesis and test anticancer drug efficacy (4). Moreover, development of mouse strains with more severe immunodeficiencies, such as NOD/SCID mice and NOD/SCID IL2rg$^{-/-}$ mice, further facilitated the use of rodents to efficiently repopulate primary human cancer samples or cells and served to mirror the heterogenous features of these cancers in patients [patient-derived xenograft (PDX) models] (5). The application of CDX and PDX models in cancer research markedly facilitates human cancer biology investigations and anticancer drug interventions. However, more recent analysis has revealed that the absence of human immune elements in these models may severely compromise their value in translational research and the development of novel human cancer immunotherapies (6).

It is widely accepted that the immune system is closely related to oncogenesis and cancer prognosis as well as response to anticancer therapies (7). Key breakthroughs in cancer immunotherapies, including co-stimulatory molecule blockade (8) and chimeric antigen T (CAR-T) cell transfer (9), further highlight the importance of including human immunity in cancer research. Humanized mice are novel animal models designed to address some of these concerns, thereby making them an attractive alternative for biomedical research (10). Briefly, humanized mice are engineered to carry human genes, cells, or tissues, allowing them to directly mirror human physiological and pathological characteristics (10). Humanized mice with functional human immune systems (HIS mice) could be a powerful model for understanding the interaction between human immune components and human cancer and contributing to anticancer intervention development (11, 12).

Here, we have focused on cancer studies that use humanized mice with functional human immune reconstitution and discuss their advantages and disadvantages and prospect their advancement in the future.

HUMANIZED MICE WITH HUMAN CANCER DEVELOPMENT

Immunodeficient Mice

Robust xenogeneic immune rejection is a major barrier to the engraftment of human cancer cells in immune-competent rodents (13). Several immunodeficient murine strains have been developed by disrupting the relevant genes crucial for immune cell development/survival/function. The ability to construct these immunodeficient animals is a cornerstone in producing humanized mice for evaluating human cancer development. The characteristics of these immunodeficient murine models have been reviewed in detail in earlier studies (10). Briefly, immunodeficient mice were designed to overcome the rejection of human cancer cells mediated by the mouse adaptive (T and B cells) and innate (NK cells and macrophages) immune responses (11). For example, elimination of the forkhead box N1 (*Foxn1*) gene (4), recombination activating gene 1 (*Rag1*) (14), recombination activating gene 2 (*Rag2*) (15), protein kinase, and DNA activated and catalytic polypeptide (*Prkdc*) genes (16) results in mice with T and/or B cell deficiency; deletion of interleukin 2 receptor subunit gamma (*IL2rg*) (17) or β2-microglobulin (*B2m*) (18) genes leads to the absence or functional impairment of mouse NK cells, whereas selection of non-obese diabetic (NOD) mouse background (19) or knock-in human (20) or NOD (21) *Sirpa* genes prevents phagocytosis by mouse macrophages. Combinations of these genetic engineering strategies have been applied to develop the popular immunodeficient mouse strains, such as NOD/*Prkdc*^scid^ (NOD/SCID), NOD/SCID IL2rg$^{-/-}$ (NSG or NOG), and Balb/c Rag1$^{-/-}$ IL2rg$^{-/-}$ (BRG) that have all been used in human oncology studies (11) (**Table 1**).

CDX and PDX Mouse Models

Based on the type of human cells or samples used in the transplantation, immunodeficient mice grafted with human cancer cells can be classified as CDX or PDX mouse models (4, 5). Following *in vitro* culture of human cancer cell lines for many passages, they can easily form human tumors in most T-cell deficient immunodeficient mouse strains, making them a valuable initial model for cancer investigation. For example, in order to study the underlying mechanisms why *BRAF* mutations are co-related with aggressive, less-differentiated, and therapy-resistant colorectal carcinoma clinically, Ricarda Herr et al. established a CDX mouse model based on human colorectal cancer cell lines whose B-Raf^V600E^ expression can be conditionally knocked down by doxycycline treatment, through which they revealed a novel facet of clinically applied B-Raf or MEK inhibitors by promoting cellular adhesion and differentiation of colorectal carcinoma cells (26). While long-term *in vitro* culture does result in the loss of many of the inherent features and heterogeneous characteristics of their parental cancer tissues. These shortcomings are most apparent when these cell lines are compared with their parental cancer strains from sick patients, implying that using these cell lines may compromise the value of any anticancer drug efficacy predictions in a clinical setting (27, 28). PDX mouse models are usually generated using mice with combined T/B/NK cell deficiency and macrophage tolerance for human cells, like NOD/SCID and NSG/NOG mice, which are repopulated with primary human cancer cells or tumor samples *in vivo*. Compared with CDX mouse models, PDX mouse models retain much more of their parental malignancy characteristics and are considered a more powerful tool for evaluating the effect of anticancer drugs in pre-clinical studies (29) (**Table 2**). For instance, Dr. Sidransky's group performed PDX studies in a large heterogeneous population (237 patients with various tumor types) and verified that human tumor grafts in PDX models can faithfully conserve genetic patterns of primary tumor. Additionally, their analysis further demonstrates that PDXs accurately replicate patients' clinical outcomes after treatments, indicating the capacity of this platform for assessment of anticancer drug efficacy (47).

Mouse Models of Spontaneous Human Oncogenesis

One major shortcoming of both the CDX or PDX models for human oncogenesis is the lack of an oncologic transformation process from normal cells into malignant cells. Transplantation of healthy human cells in which tumor-suppressive genes were disrupted and/or oncogenes were overexpressed into immunodeficient mice has been used to simulate the entire oncogenesis process for a number of cancers, including leukemia, lymphoma, and melanoma (1) and so on (**Table 3**). For example, mice with spontaneous human acute human B lymphoblastic leukemia (B-ALL) were created by transplanting human CD34$^+$ hematopoietic stem cells (HSCs) after transduction with retroviral vectors carrying *MLL-AF9* genes into immunodeficient mice, allowing the researchers to evaluate the underlying mechanisms of human B-ALL development (52). Similarly, seeding human melanocytes transformed with mutated melanoma-associated genes, including *N-Ras*^G12V^, *CDK4*^R24C^,

TABLE 1 | Immunodeficient mouse strains for human cancer study.

Name	Strain	T	B	NK	Macrophage (for human cells)	Complement	References
Nude	Foxn1^{null}	No	Yes	Yes	Phagocytose	Yes	(4)
Scid	B6.CB17-Prkdc^{scid}/SzJ	No	No	Yes	Phagocytose	Yes	(22)
BRG	BALB/c.Rag2$^{-/-}$ IL-2Rg$_c^{-/-}$	No	No	No	Partial tolerant	Yes	(11)
NOD-scid	NOD.CB17-Prkdc^{scid}/J	No	No	Function impaired	Tolerant	No C5	(23)
NOD/SCID B2m^{null}	NOD.Cg-B2m^{tm1Unc}Prkdc^{scid}/SzJ	No	No	Function loss	Tolerant	No C5	(18)
NSG	NOD.Cg-Prkdc^{scid}IL2rg^{tm1Wjl}/SzJ	No	No	No	Tolerant	No C5	(24)
NOG	NOD.Cg-Prkdc^{scid}IL2rg^{tm1Sug}/JicTac	No	No	No	Tolerant	No C5	(25)
BRGS	BALB/c.Rag2$^{-/-}$IL-2Rg$_c^{-/-}$NOD.sirpa	No	No	No	Tolerant	Yes	(21)
hSIRPa-BRG	BALB/c.Rag2$^{-/-}$IL-2Rg$_c^{-/-}$human.sirpa	No	No	No	Tolerant	Yes	(20)

TABLE 2 | Advantages and applications of PDX mouse models for cancer study.

Cancer	Advantages	Applications	References
Lung cancer	Retain genetic and histological characteristics.	Predict the possibility of relapse after curative surgery.	(30)
Colorectal cancer	Retain the intratumor clonal heterogeneity and chromosomal instability.	Predict responsiveness to cetuximab in patients.	(31, 32)
Pancreatic cancer	Maintain the original tumor architecture; retain a greater proportion of stromal components and develop locoregional and distant metastases.	Demonstrate the activity of mitomycin C and cisplatin in a patient harboring a *PALB2* mutation. Demonstrate that stromal modulation may increase intra-tumor gemcitabine concentrations to improve therapy efficacy.	(33–35)
Head and neck cancer	Highly reflect promoter methylation in tumors and reproduced tumor heterogeneity.	Predict phase II clinical drug activity of cisplatin, diaziquone, pazelliptine, and retelliptine.	(36)
Breast cancer	Retain basal-like morphology and tumor structure.	Demonstrate the activity of cisplatin and ifosfamide combinatory therapy; evaluate the efficacy of trastuzumab.	(37, 38)
Glioblastoma multiforme	Retained genetic characteristics.	Assess the efficacy of bevacizumab.	(39, 40)
Renal cell carcinoma	Maintain the ability to evaluate tumor angiogenesis; Retain genetic and histological characteristics.	Evaluate the effects of sorafenib or sunitinib.	(41–43)
Prostate cancer	Exhibit the differentiation and expression of androgen receptor and prostate-specific antigen (PSA).	Predict the efficacy of androgen ablation therapy.	(44, 45)
Melanoma	Retain histology, genetic profiles, and tumor antigen characteristics.	Treatment with temozolomide exhibits similar responses to the corresponding patients.	(46)

and dominant-negative $p53^{R248W}$, which are critical for p16^{INK4A}-CDK4-Rb and ARF-HDM2-p53 tumor suppressor pathways, into autologous human skin grafts in immunodeficient mice results in the development of human melanocytic neoplasia *in vivo*, demonstrating the value of mouse models in the functional analysis and validation of mutations observed in human melanoma (53).

Non-immune Factors Affect Human Oncogenesis in Mouse Models

Other than immunological factors, non-immune factors related to tumor-associated micro-environments may also influence the feasibility and quality of using mouse models to study human oncogenesis, especially for some human hematological malignancies (54). Aberrant gene expression in different stages of human HSC differentiation leads to a variety of hematological malignancies, including B cell leukemia, myeloid leukemia, and myeloma (55). The lack of or suboptimal interaction with mouse cytokines/chemokines/ligands, which are crucial for human hematological differentiation or cell survival may impair human oncogenesis in recipient mice

(54). For example, poor interaction between mouse GM-CSF/IL-3 and IL-6 and human cells reduces the practicality of using immunodeficient mice to recapitulate human myeloid leukemia (56) and myeloma (57), respectively. The generation of immunodeficient mice with relevant human cytokine expression markedly improves their value in investigating aberrant human hematological complications, including acute myeloid leukemia (AML) (56), myeloma (57), chronic myelomonocytic leukemia (CMML) (58), juvenile myelomonocytic leukemia (JMML) (58), and myelodysplastic syndromes (MDS) (59). In addition, "humanization" of mouse bone marrow micro-environments by adding human stromal cells also facilitates the pathogenesis of human hematological disorders. For instance, Dr. Daniel Nowak's group showed that intra-bone injection of MDS patient-derived mesenchymal stromal cells (MSCs) contributes to the propagation of MDS-initiating stem cells and disease progression in orthotopic xenografts of NSG and NSG-SGM3 animals (NSG animals constitutively expresses human GM-CSF/IL-3 and stem cell factors) (60). Majeti et al. reported that an artificial human bone marrow (BM) microenvironment can be constructed by the subcutaneous injection of human BM-derived MSCs (humanized

TABLE 3 | Spontaneous human cancer model.

Cancer	Method	References
Myeloid neoplasia	Using CRISPR/Cas9 technology to introduce *FLT3-ITD* and *SMC3* mutation in CD34$^+$ cells, and transplant them to NSG mice.	(48)
Lung cancer	Incorporate the mutated genes (*CDK4, hTERT, sh-p53, KRASV12,* and *c-MYC*) by lentiviral vectors into human bronchial epithelial cells, and transplant them to NOD/SCID mice.	(49)
T-ALL	Incorporate *NOTCH1*ΔE (N) and *LMO2/TAL1/BMI1* (LTB) gene by lentiviral vectors to into CD34$^+$ cells, and transplant them to NSG mice.	(50)
AML	Incorporate *BCR-ABL* gene into CD34$^+$ umbilical blood cells by retroviral vectors, and transplant them to NSG mice.	(51)
B-ALL and AML	Incorporate *MLL-AF* gene into CD34$^+$ cells by retroviral vectors, and transplant them to BRG mice.	(52)
Melanoma	Incorporate *N-Ras*^{G12V}, *CDK4*^{R24C}, and dominant-negative *p53*^{R248W} into human melanocyte by retro-viral vectors, and transplant into the autologous human skin graft in CB.17 scid mice.	(53)

TABLE 4 | Improvement of mouse models for human hematological malignancy study.

Strategy	Cancer	Model construction	References
Human cytokine expression	MDS	Newborn MISTRG mice were intrahepatically injected with split-donor MDS BM CD34$^+$ cells.	(59)
	AML	Newborn MISTRG mice were intrahepatically injected with primary favorable-risk AML cells.	(62)
	CMML	NSG-SGM3 mice were intravenously injected with CD34$^+$ cells sorted from CMML patients' bone marrow or peripheral blood.	(58, 63)
	JMML	NSG-SGM3 mice were intravenously injected with CD34$^+$ cells sorted from JMML patients' bone marrow or peripheral blood.	(58)
Human MSCs implantation	AML	Inject primary human AML cells into human BM-MSC formed ossicle in NSG mice.	(61)
	APL	Inject primary human APL cells into human BM-MSC formed ossicle in NSG mice.	(61)
	MDS	Human CD34$^+$ cells and MSCs collected from MDS patients were simultaneously intra-bone injected into NSG mice.	(60)

ossicles), which enables robust engraftment of healthy human HSCs as well as primary human leukemia-initiating cells from AML, acute promyelocytic leukemia (APL), and myelofibrosis (MF) (61) (**Table 4**).

HUMANIZED MOUSE MODELS WITH HUMAN IMMUNE SYSTEMS IN CANCER RESEARCH

It is widely accepted that immune surveillance is closely involved in oncogenesis and has a significant impact on treatment efficacies and outcomes (7). Additionally, the successful application of cancer therapeutic regimens, including co-stimulation signal blockades and adoptive transfers of anticancer immune subsets in treating metastatic malignancies, which show poor prognosis using traditional therapies, further highlights the importance of human immunity in the investigation of human oncology (8, 64). Therefore, humanized mice reconstructed with human immune systems are expected to aid the comprehensive study of the interactions between human cancer and human immunological elements.

Humanized mice with human immune systems have been extensively studied over the past three decades and have been reviewed in detail in several previous reports (10–12, 54). Various humanized mouse models, including the Hu-PBL (peripheral blood leukocyte)-SCID model (65), SRC (SCID repopulating cell)-Hu model (17, 66), and Thy/HSC (23, 67) [also named as BLT (68)] model, are commonly used in human oncology studies with each model having their own unique advantages and disadvantages (**Table 5**).

Hu-PBL-SCID Model

The Hu-PBL-SCID model is created by injecting human PBLs into immunodeficient mice, which transiently host multi-lineage human immune subsets (65). However, due to the lack of self-renewing human hematopoietic stem/progenitor cells and the relatively short life span of mature immune subsets, limited numbers of human myeloid cells and B cells engrafted in these mice. Instead, engrafted human immune cells primarily belong to activated human CD4$^+$ or CD8$^+$ T cell driven by mouse major histocompatibility (MHC) molecules, which cause severe xenogeneic graft vs. host diseases (xeno-GVHD), thereby restricting the experimental window for these animals to few weeks (69). Due to the relatively simple handling and accessibility of human PBL samples, the Hu-PBL-SCID model is widely used to study interactions between human immune cells, including T cells and NK cells, and human tumors *in vivo*. For example, Jakobsen et al. reported the feasibility of using a Hu-PBL-SCID model to evaluate the efficacy of a Bi-specific TCR (T cell receptor)-anti-CD3 regimen for treating NY-ESO-1- and LAGE-1-positive human tumors (70); Ignacio Melero et al. showed that this model can be used to study the effects of human PD-1 (Nivolumab) and CD137 (Urelumab) antibodies on the T cell-mediated anti-tumor response *in vivo* (71). Interestingly, Ryuzo Ueda et al. reported that Hu-PBL-SCID can also be used to study the anti-tumor effects driven by human NK cells through antibody-dependent cellular cytotoxic (ADCC) approaches (72). To overcome the constraints of xeno-GVHD, researchers went on to develop MHC knockout immunodeficient mice, like NOG-dKO or NOG-β2 m, IAβdKO mice, which demonstrate a reduced susceptibility to xeno-GVHD and extended experimental time frames (73, 74).

SRC-Hu Model

The SRC-Hu model with human immunity is usually constructed by transplanting human hematopoietic stem/progenitor cells into neonatal or adult Il2 rg knockout immunodeficient recipients, like NOG (17, 75), BRG (66), NSG (76), or other similar murine strains. Here, mice present with a sustainable human

TABLE 5 | Comparison between different HIS humanized mouse models.

	Hu-PBL-SCID	SRC-Hu	Thy/HSC (BLT)
Accessibility of human sample	Good	Moderate	Difficult (potential ethic problem)
Technique for model construction	Easy	Moderate	Relative difficult (required anesthesia and transplant technique)
Human immune cell survival/development	Majority of activated human T cells; Transient human B cells, myeloid cells and NK cells;	Multi-lineage human immune cell reconstitution; Poor human thymopoiesis; Lack of HLA mediate thymic selection for human T cells.	Multi-lineage human immune cell reconstitution; Robust human thymopoiesis; Human TCR repertoire influenced by mouse antigen.
Human immune function	T cell responses; Lack of interaction between human T cells, B cells, and myeloid cells.	Poor HLA restricted T cell responses; Poor T cell-dependent humoral responses.	Good HLA restricted T cell responses; Good T cell-dependent humoral response.
Time window	Short	Long	Long

immune system composed of human T cells, B cells, and myeloid cells. Due to the accessibility of cord blood and the relatively easy construction procedure, the SRC-Hu mouse model is one of the most popular humanized mouse models for the research of human immune relevant subjects (11). The SRC-Hu model is a valuable tool to be used to evaluate co-stimulatory molecule blockade effects and study anticancer drug effects in a physiologically relevant immune environment (77). For instance, Wang et al. showed that PD-1-targeted immunotherapy can be modeled in SRC-Hu humanized NSG mice, but not control NSG mice, bearing CDX and PDX partial HLA matched human tumor (referred to human immune system), demonstrating the value of the SRC-Hu model for cancer immunotherapy investigation (78). However, human T cells are educated in the mouse thymus and these animals have very poor human thymopoiesis (75). In addition, SRC-Hu mice cannot efficiently generate HLA-restricting antigen-specific immune responses (79). These limitations may restrict the value of the SRC-Hu model in human immune-oncology studies.

Thy/HSC Model

In 2004, based on the Dr. McCune's Thy/Liv SCID-Hu mouse model (80), our group developed a novel humanized mouse model by co-transplantation of human fetal liver and thymic tissues into the renal capsule and intravenous injection of CD34$^+$ human fetal liver cells (FLCs) into NOD/SCID mice after total body sublethal irradiation (67). These humanized mice have high levels of multilineage human lymphohematopoietic cell reconstitution, including human T cells, B cells, conventional dendritic cells (cDCs), plasmacytoid dendritic cells (pDCs), and macrophages, which can be detected in the blood as well as the lymphoid organs (23). Later, we found that human fetal liver tissue implantation is dispensable for human immune system reconstitution in this humanized mouse model, and named it as the Thy/HSC model (12). This method of construction was confirmed by Dr. J. Victor Garcia and colleagues in 2006 who named them BLT mice (68). Unlike the SRC-Hu model, robust human thymopoiesis occurs in engrafted human thymic tissues, in which typical thymic structures including cortex, medulla, and Hassall's bodies are formed by human stromal

cells and human T cell progenitor cells can be identified (23). Importantly, Thy/HSC humanized mice can generate potent human immune responses as evidenced by the capability of these animals to reject allogeneic (81) and xenogeneic grafts (82), their generation of HLA-restricted antigen-specific human T cell reactions, and their ability to produce antigen-specific human IgM and IgG antibodies with subclass switching after immunization or xenograft implantation (82, 83). In Thy/HSC mice, mouse dendritic cells can migrate into human thymic grafts and may play a role in human T progenitor cell thymic education, thus explaining the reduced incidence of xeno-GVHD syndrome in Thy/HSC mice. Moreover, cryopreservation and "pipetting" of human thymic grafts before transplantation can eliminate existing human T cell progenitor cells, further alleviating xeno-GVHD syndrome, with some animals only experiencing xeno-GVHD more than 25 weeks post-transplant (84–86). For these reasons, Thy/HSC models are considered one of the most powerful *in vivo* tools for investigating human immune responses and their effects on therapeutic interventions (87) and are already widely applied in many biomedical fields including pathogen infection (88), allo/xeno-transplantation (81, 82), autoimmune diseases (89), regenerative medicine (90), immune molecule-targeting drug tests, and cancer research (91). For example, Thy/HSC mice can be modified to act as TCR transgenic humanized mice to study human T cell adaptive immunotherapy (92). Briefly, a melanoma antigen (MART-1)-specific TCR transgenic humanized mouse model was constructed by co-transplanting HLA-A*0201$^+$ human fetal thymic tissues and autologous human CD34$^+$ FLCs transduced with lentiviral vectors containing HLA-A*0201 restricted MART-1 specific TCR genes into sub-lethal irradiation pre-conditioned NSG mice, in which most MART-1-specific T cells contained only MART-1 TCR alpha and beta chains due to the allele exclusion process in the human thymic grafts (93). Using this tool, we revealed that the simultaneous inclusion of rapamycin for MART-1 TCR$^+$ human T cell expansion *in vitro* and supplementation with human IL-15 *in vivo* greatly improves the anti-melanoma effects mediated by adoptive transfer of human MART-1 TCR$^+$ T cells (92).

One of the main drawbacks of the Thy/HSC model is the requirement of human fetal samples for model construction,

which are difficult to obtain by researchers due to ethnic problem and/or local policy (94). In addition, the TCR repertoire of human T cells generated in Thy/HSC mice may be different from the ones in human, because of the involvement of mouse antigen in human thymic educating process in Thy/HSC humanized mice.

# IMPROVING HUMAN IMMUNITY IN HUMANIZED MICE FOR CANCER RESEARCH

Despite the fact that high human lymphohematopoietic chimerism can be constructed in Thy/HSC and SRC-Hu mice, there are still several limitations in the combination and functionality of the human immune system in humanized mice when compared with humans, which are primarily caused by the "unfriendly" mouse microenvironment for human immune cells to survival/differentiate/migrate/function (54). For example, there are significantly lower levels of myeloid cells and NK cells in humanized mice compared to human due to a lack of sufficient cross-talk between the mouse cytokines (GM-CSF/IL-3, M-CSF, Flt-3-l, IL-15, etc.) and the human cells, which limits their application in the study of the interactions between human myeloid/NK cells and human tumor tissues (54). Human immunity in humanized mice can be improved by supplementing the corresponding human cytokines by injecting recombinant proteins (95) or hydrodynamic injections of plasmids containing human cytokine genes (96) or using immunodeficient transgenic mice expressing the relevant proteins (97). Continuous overexpression of human cytokine (such as NSG-SGM3) may lead to abnormal human cell function; thus a series of human cytokine knock-in (human gene driven by mouse promotor) immunodeficient mouse strains (such as MISTRG) were developed to ensure an appropriate tissue-, cell-, and context-specific expression of the incorporated protein (98–100). These strategies greatly expand the application of these humanized mice in immune and cancer research. For example, Dr. Richard Flavell's group showed that humanized mice made using MISTRG (human M-CSF, GM-CSF/IL-3, TPO knock in $Rag2^{-/-}Il2rg^{-/-}$ mice with transgenic human *Sirpa* expression) mice showed significantly improved cellular ratios as well as function of human myeloid and NK cells in blood and lymphoid organs when compared to $Rag2^{-/-}Il2rg^{-/-}$ or NSG mice (100). In addition, they also showed that human macrophage infiltration in a human tumor xenograft in MISTRG humanized mice used a similar mechanism to those exhibited in human tumor biopsy samples (100). In another report, they showed that humanized SRG-15 mice (knock-in with human IL-15 and *Sirpa* gene in $Rag2^{-/-}Il2rg^{-/-}$ mice) have dramatically improved NK cell development and function. These cells demonstrate similar expression patterns of killer inhibitory receptors when compared to NK cells from human donors, which is an important breakthrough in the application of humanized mice in the study of human NK cell-mediated and combinatory cancer immunotherapy strategies *in vivo* (20).

Although development of human cytokine transgenic/knock-in immunodeficient mice optimizes human immune function, researchers must still be aware of the potential interference resulting from the persistent secretion of these inflammatory human cytokines in recipient mice. Our recent studies have shown that Thy/HSC humanized mice made on NSG-SGM3 but not NSG mice showed dramatically reduced life spans and increased incidence of hemophagocytic lymphohistiocytosis (HLH) syndrome, as evidenced by elevated levels of human inflammatory cytokines, including IL-6, IL-4, IL-10, IFN-γ, TNF-α, IL-18, severe anemia/thrombocytopenia phenomena, and aberrant activation and infiltration of human macrophages and T cells in systemic organs (85). Similar phenomena were also observed in SRC-Hu models made using NSG-SGM3 (101) mice and MISTRG mice (102). Bondanza et al. reported that CAR-T cells induced cytokine-release syndrome (CRS) and neurotoxicity caused by the secretion of IL-1 and IL-6 by human monocytes can be modeled in humanized mice made using NSG-SGM3 mice but not in NSG mice, which also highlights the important role of inflammatory cytokine secretion in animal mortality and aberrant human immune activation (103). Thus, interpretation of the results collected from humanized mice made using immunodeficient recipients with abnormal human cytokine secretion may need to consider these factors.

HUMANIZED MOUSE MODELS OF HUMAN ONCOGENESIS UNDER AUTOLOGOUS HUMAN IMMUNE SURVEILLANCE

One major limitation of most humanized mouse models created for human cancer study is that these reconstructed human immune systems are allogeneic to the inoculated human tumors (102). Thus, robust allogeneic responses conferred by human T cells to human tumors may compromise the value of the data collected from these models, making it difficult to extend the observations from these humanized mice to the complex interactions between the tumor antigen-specific T cells and human tumor tissues in patients, or precisely predicting anticancer drug effects (102). As such, the development of humanized mouse models in which human oncogenesis occurs under autologous functional human immune surveillance is critical to the field's continued development (**Figure 1**).

Recently, we developed a humanized mouse model with spontaneous development of human B-ALL under autologous human immune surveillance by incorporating the leukemia-associated fusion gene, *MLL-AF9*, into human CD34+ FLCs, which were then co-transplanted with human fetal thymus tissue into NSG mice (104). Human B-ALL collected from these humanized mice can survive and expand into the Thy/HSC humanized mice made by autologous but not allogeneic human fetal samples. Using this model, we were able to show that recipient leukocyte infusions (RLI), a GVHD-free immunotherapeutic approach, markedly reduced human leukemia burden during induced lymphopenia, further validating the safety and efficacy of human RLI for human leukemia

FIGURE 1 | Application of humanized mouse models with human oncogenesis under autologous human immune surveillance for cancer research.
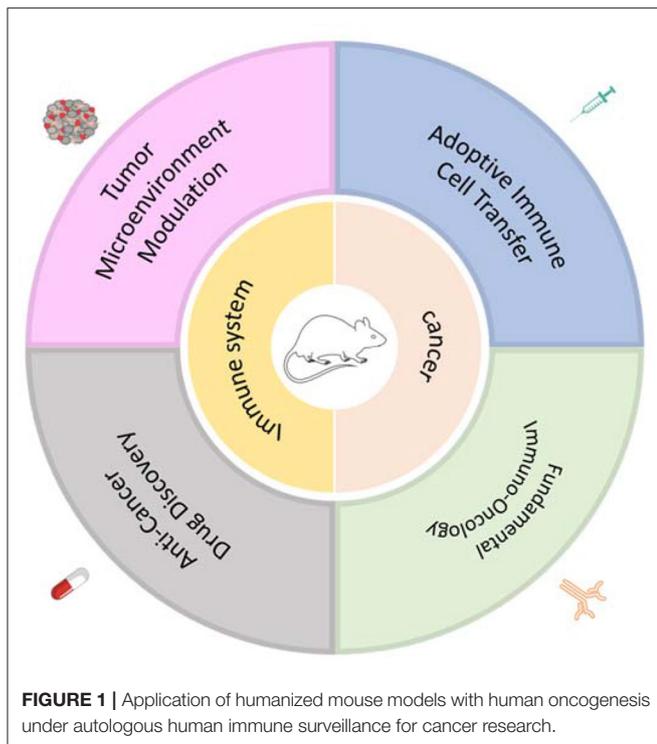
treatment (104). Moreover, this model can also be utilized to study chimeric antigen T (CAR-T) cell therapy, which still requires modification to improve anticancer function while restricting various side effects, including CRS and neurotoxicity. We showed that Thy/HSC mice with autologous B-ALL treated with anti-CD19 CAR-T cells exhibited similar kinetics and levels to those observed in patients, and rapid production of T cell- and myeloid cell-derived cytokines, such as GM-CSF, IFN-γ, TNF-α, and IL-10, and elevation of regulatory T cell frequency, which has been reported in patients receiving CAR-T therapy, were also found in this mouse model (105). These results indicate that these animal models could be reliably used to characterize human CAR-T cell function *in vivo* and facilitate the development of novel CAR therapies.

In addition, a novel humanized mouse model, named PDXv2.0, was recently constructed by Dr. Jonas A. Nilsson's group through adoptive transfer of *in vitro* expanded human tumor infiltrating lymphocytes (TILs) into the PDX mice that host the tumor collected from the same patients (106). They showed that the PDX2.0 mouse model made by immunodeficient mouse recipients with human IL-2 continuous production efficiently represents the reactivity of adoptive cell transfer (ACT) immunotherapy that occurred in patients, offering a powerful platform to model ACT-based immunotherapies as well as combinatory therapies for heterogeneous human cancers (106). Based on a similar model, Dr. Ignacio Melero et al. verified that transient expression of IL-12 mRNA in human antitumor CD8$^+$ T cells by electroporation can markedly improve their antitumor effects after intratumor adoptive transfer (107).

DISCUSSION AND CONCLUDING REMARKS

When compared to traditional rodent models, humanized rodents provide a much closer approximation of human physiology and pathology with broader application in basic cancer research and anticancer drug/approach discovery. While more effective methods of construction, design, and functionality for these models will still be required to make them able to address specific concerns and boost translation of basic cancer research. We hypothesize that the optimization of humanized mice in cancer research may result from the following scenarios: (1) Invention of humanized mouse models with human immune systems and autologous human oncogenesis for more types of human tumors including melanoma, lung cancer, hepatocarcinoma, and colorectal cancer, in which pluripotent stem cell technology (108) and gene editing tools, such as CRISPR/Cas9, may play crucial roles (109); (2) Development of humanized mouse models with functional human adaptive and innate immunity that do not require human fetal or even cord blood samples, to reduce ethical considerations and broaden their application (94, 110); for example, substitution of human fetal thymic tissue with HLA transgenic porcine thymic tissue (111) to make Thy/HSC models or the generation of humanized mice using pluripotent stem cell-derived human hematopoietic stem/progenitor cells (112) and human thymic epithelial cells (113); (3) Development of immunodeficient mouse strains with relevant human cytokine/chemokine/ligand secretion under physiological conditions to promote reconstructed human immunity without aberrant human immune disorder; (4) Establishment of humanized large animal models, such as humanized pig models, in which human cancer therapy could be modeled at more physiologically relevant scales and closer physiological conditions using more relevant timelines (114); (5) Generation of personalized humanized mouse models (86) that can simultaneously host primary human cancer samples and reconstitute the patients' unique immune system as a personalized platform to substitute patients for anticancer therapy tests.

In summary, humanized mouse models with optimized designs could offer a more powerful tool to not only better understand the roles of human immune elements in human cancer development and treatment but also facilitate the invention and translation of novel anticancer therapeutic drugs/approaches in the future.

AUTHOR CONTRIBUTIONS

HT and YL performed literature search and wrote the manuscript. Y-GY and ZH conceived the framework of this article, wrote, and edited the manuscript. All authors contributed to the article and approved the submitted version.

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