Humanized (SCID) Mice as a Model to Study human Leukemia

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A humanized mice (hu-mice) model is extremely valuable to verify human cell activity *in vivo* condition and is regarded as an important tool in examining multimodal therapies and drug screening in tumor biology. Moreover, hu-mice models that simply received human CD34⁺ blood cells and tissue transplants are also overwhelmingly useful in immunology and stem cell biology. Because generated hu-mice harboring a human immune system have displayed phenotype of human CD45⁺ hematopoietic cells and when played partly with functional immune network, it could be used to evaluate human cell properties *in vivo*. Although the hu-mice model does not completely recapitulate human condition, it is a key methodological factor in studying human hematological malignancies with impaired immune cells. Also, an advanced humanized leukemic mice (hu-leukemic-mice) model has been developed by improving immunodeficient mice. In this review, we briefly described the history of development on immunodeficient SCID strain mice for hu-and hu-leukemic-mice model for immunologic and tumor microenvironmental study while inferring the potential benefits of hu-leukemic-mice in cancer biology.

Key Words: Humanized, Mice model, Acute myeloid leukemia

INTRODUCTION

The humanized mice (hu-mice) model is commonly reconstituted with human blood-derived CD34⁺ stem cells using immune compromised mice such as SCID (Bosma et al., 1983) and Rag^{null} strain mice (Mombaerts et al., 1992). An advanced hu-mice model has already been developed by increasing the defected function of immune cells and hu-mice with impaired natural killer cells is an emerging innovative model to study human diseases. The hu-mice

model is mainly divided into four classes including NOD/LtSz-*scid* IL2R γ^{null} (termed NSG, generated from Jackson Lab), NODShi.Cg-PrkdcscidIL2R γ^{tmunc} (termed NOG, generated from Central Institute for Experimental Animals), BALB/c-Rag2^{null}IL2R γ^{null} (termed BRG, generated from Yale Univ.) and Stock-H2^d-Rag2^{null}IL2R γ^{null} (termed H2^dRG, generated from Pasteur Institute) (Shultz et al., 2007). Because these mice display a long life span, impaired innate/adaptive immunity, and engage in the development of hematopoietic lineage cells of donors, these immunodeficient mice are regarded as having a strong strain to sustain human cell engraftment. Since NOD/SCID mutated mice from the CB17-*scid* mice can allow superior engraftment of human cells (Shultz et al., 1995) than nude and SCID mice, the hu-mice model using NOD/SCID and Rag2^{null}IL2R γ^{null} strain mice has been used recently due to loss of murine immune network in immunology fields (Goldman et al.,

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1998; Kollet et al., 2000; Ishikawa et al., 2005). This progression in the hu-mice model can be used as an effective tool in understanding normal human hematopoiesis including immune cells *in vivo* (Lubin et al., 1991; Lapidot et al., 1992; Huntington et al., 2009). Amid many biomedical researches, the hu-mice model can extremely facilitate the study of hematopoietic malignancies such as acute myeloid leukemia (AML). Because AML is heterogeneous and because complex issue with impaired immune system and disease prognosis is thoroughly variable, data from *in vitro* or *ex vivo* in AML have limitations in mimicking and understanding the natural propagation of AML, so the establishment of effective humanized leukemic mice (hu-leukemic-mice) model is a priority in AML study (Sawyers et al., 1992; Lee et al., 2014). Indeed, results from a culture dish cannot fully represent leukemogenic events in a micro-environment. Thus, immune compromised hu-leukemic-mice model is regarded as more precise tool to investigate relevance between the immune cells and HSCs/LSCs in presenting patient's environment. Since Mercier FE et al. highlighted the importance of communication between immune cells and hematopoietic cells in bone marrow (BM) indicating microenvironmental orchestration (Mercier et al., 2012), immune crosstalk with hematopoiesis and leukemogenesis is an emerging issue in microenvironmental biology. Microenvironmental domains in leukemia possess cellular components including osteoblasts, reticular cells, endothelial cells, mesenchymal stromal/stem cells, immune cells as well as non-cellular cytokines (Kfoury and Scadden, 2015; Kim et al., 2015). Especially, chemotherapy-resistant leukemic cells are typically able to change niche compositions, including in immune and stromal cells. Their mutual interaction can cause leukemia to adversely develop without blast apoptosis (Konopleva et al., 2002) or directly suppress the relapse of leukemia by enhancing of immune cells cytotoxic machinery (Zheng et al., 2012). Hu-leukemic-mice model is absolutely necessary to figure out the AML *vivo* phenomenon with acquiring confident evidence. Here, we briefly summarized hu-or hu-leukemic-mice models in SCID strain with injection of human cells and suggest the necessity of hu-mice models in biomedical research, especially for leukemia.

Development of hu-mice model

Since the generation of the CB17-*Prkdc*^{scid} severe combined immunodeficiency (SCID) mice model by Bosma GC et al., improvements of hu-mice model have been successively developed (Bosma et al., 1983). In 1988, Namikawa R and Mosier DE et al. first reported the injection of human immunodeficiency virus-1 and human peripheral blood leukocytes into SCID-hu mice, respectively (Mosier et al., 1988; Namikawa et al., 1988). Generation of SCID has been established in Balb/c, B6, B17, and AKR backgrounds and SCID model can provide high efficacy in grafting human cells, compared to that of nude mice. In 1988, both Weissman IL and Wilson DB groups published that human fetal liver derived hematopoietic cells and peripheral blood leukocyte (PBL) cells injected into SCID mice, respectively (McCune et al., 1988; Mosier et al., 1988). However, Weissman IL group failed to show functional immune network and only presented human phenotype in PB status. Leukocytes contain rare population for stem cells, which can produce all hematopoietic cells lineages, and show severe variation in reproducing human cell engraftments (Mosier et al., 1988). Meanwhile, human stem cells enriched BM was first used into irradiated Balb/C mice by Lubin I et al (Lubin et al., 1991). Because irradiation partly plays a role in frustrating murine hematopoietic cells, the irradiated condition is similar to SCID mice. However, immune reconstitution after transplantation in irradiated Balb/C was delayed and displayed low efficacy for immune phenotype. At 1992, Lapidot T et al. used SCID strain and reported that a stable hu-mice model using BM cells with IL-3 and GM-CSF was established and that it can allow immature human hematopoietic cells, implying that the disease model is available (Lapidot et al., 1992). Nevertheless, a disadvantage of the SCID strain found that it is the low frequency of functional immune cells. Also, engraftment efficiency is higher in human tumors or viruses rather than in human primary cells. To overcome these issues, the development of immunodeficient non-obese diabetic (NOD) /SCID mice as a second breakthrough was advanced (Shultz et al., 1995). Natural killer (NK)-cell activity, which is a main

obstacle in the engraftment of human HSCs (Christianson et al., 1996), was lower and HSC engraftments were higher in NOD/SCID than in SCID mice (Shultz et al., 1995).

Despite constantly being advanced in the NOD/SCID model, drawbacks still remain to be resolved such as the low efficiency of engraftments of human cells, short life span,

	Year	PMID	Journal, Author	Cell sources & used mouse strain
(1)	1983	6823332	Nature, Bosma GC <i>et al</i>	CB17SCID mice generation
(2)	1988	3201256	Science, Namikawa R <i>et al</i>	HIV to SCID
(3)	1988	2970594	Nature, Mosier DE <i>et al</i>	Peripheral blood Leukocyte into SCID
(4)	1992	1372131	Science, Lapidot T <i>et al</i>	BM with IL-3 to SCID
(5)	1995	7995938	J Immunol, Shultz LD <i>et al</i>	Hematopoietic cells into NOD/SCID
(6)	1995	7717456	Am J Pathol, Greiner DL <i>et al</i>	Spleen to NOD/SCID
(7)	2000	10807775	Blood, Kollet O <i>et al</i>	Cord blood mononuclear cells to NOD/SCID/B2m _{null}
(8)	2002	12384415	Blood, Ito M <i>et al</i>	CD34+ Stem cells to NOD/SCID/ γ (c) _{null} (NOG mice)
(9)	2005	15879151	J Immunol, Shultz LD <i>et al</i>	Hematopoietic stem cells to NOD/SCID/IL2R γ _{null} (NSG mice)
(10)	2005	15920010	Blood, Ishikawa F <i>et al</i>	Cord blood cells to NOD/SCID/IL2R γ _{null}

Fig. 1. History of hu-mice model. SCID generation and NOD/SCID generation leads to advanced NOD/SCID/IL2R γ _{null} mouse, which induce depletion of innate natural killer cells as well as B, T lymphocytes.

the frequent occurrence of thymic lymphoma, and residual NK cell activity. Thus, the third breakthrough mice model was focused on interleukin-2 receptor (IL-2R) γ chain (Ito et al., 2002; Ishikawa et al., 2005; Shultz et al., 2005). Ishikawa F et al. first showed newborn NOD/SCID/IL2R γ^{null} mice using CD34 $^{+}$ HSC results in complete hematopoietic system containing platelets and red blood cells (Ishikawa et al., 2005). IL-2 receptor is a pivotal receptor for IL-2, IL-4, and IL-7 ligands to improve immunologic activation and in the development of NK cells and T, B lymphoid lineage cells (Cao et al., 1995; DiSanto et al., 1995; Ohbo et al., 1996). By depletion of the IL2R γ chain, improved phenotype was observed such as the loss of thymic lymphoma and absolutely down regulated NK activity. The completion of this model in the development of hu-mice models is remarkable proficiency. In addition, modification of stem cell sources including CD34 $^{+}$ BM, PB cells, cord blood derived cells, and spleen cells was also attempted to produce more effective hu-mice models (Humeau et al., 1999; Lee et al., 2014). History of hu-mice model is briefly presented by Fig. 1.

Development of hu-leukemic mice model

AML is one of the hematologic malignancies, which occurs by immature blast accumulation with dysfunctional immune network (Lion et al., 2012; Lee et al., 2014). Additionally, uncontrolled blast proliferation results in abnormal hematopoietic cells in BM and PB (Ferrara and Schiffer, 2013). The properties of leukemia cells and the dynamic interaction of leukemia cells in a microenvironment, otherwise denoted as a "niche", is closely comprehended by the hu-mice model, but not through vitro experiments. Although an AML patient's derived cells can be isolated and studied *in vitro*, there are many unsolved questions that remain to be addressed due to unexpected individual variations. To overcome this weakness, the hu-mice model was first applied to study AML. AML cells and human leukemic promyelocytes of the HL-60 lines were subcutaneously injected into nude mice (Palu et al., 1979; Potter et al., 1984), which suppressed the number of T cells due to an absent thymus (Pantelouris, 1968). Despite the useful benefits

of nude mice, it is not enough to develop a proper hu-leukemic-mice model due to vitalization of innate immune cells. Thus, AML patient derived primary cells were applied to SCID strain mice by De Lord C (De Lord et al., 1991) several years later after SCID mice were generated by Bosma GC et al. (Bosma et al., 1983). Because the cells were intravenously implanted into irradiated SCID mice, a model is a more preferential situation with leukemic condition. However, this study only showed that human AML cells are higher in engraftment than in HL-60 cell lines, but both primary AML cells and cell lines still sustain low frequency of human cells in PB less than 9%. Unlike in AML, acute lymphoid leukemia (ALL) patients derived from primary cells displayed high rates of engraftment, suggesting variation of model stabilization based on cell sources (De Lord et al., 1991). Many attempts have been made to develop hu-leukemic-mice models with stable engraftment of human cells. For instance, a leukemia induced hu-mice model can be accessible to direct investigation in human tissues such as through secondary/tertiary transplantation of leukemic stem cells (LSCs) and niche study. Ever since embryonic rest theory was formulated in 1867 by Julius Cohnheim, stating that cancers are derived from rest embryonal cells in adult tissues, various approaches have been carried out to identify LSCs using hu-leukemic mice model. BM is one of the representative tissues containing LSCs, which also leads to the relapse of leukemia. To understand LSCs, studies of a leukemic microenvironment should be entailed with LSCs interaction. Recapitulated environments such as human bone fragments and BM cells were applied into SCID mice and revealed that biologic features of the leukemia guaranteed better than previously. Similar to normal hematopoietic stem cells, the frequency of leukemic stem or initiating cells is rare and most AML cells can rapidly lose their proliferative potential, when blasts free from niche. To identify LSCs, Lapidot T et al. first addressed the CD34 $^{+}$ CD38 $^{-}$ population as putative leukemic initiating cells using the SCID mice model (Lapidot et al., 1994). This result strongly provides the possibility that hu-leukemic mice are only available as a model to identify bona fide LSCs. As expected, SCID mice could express mature lymphocyte meaning leakiness, which is one of the

reasons for SCID defect. Because residual lymphocytes cannot allow engraftment of leukemic stem cells, some investigators performed irradiation to remove murine immune cells (Shpitz et al., 1994). These efforts partly led to in-

crease short-term engraftment of human cells, unfortunately, recipient murine die early on in the experimental schedule. Thus, Bonnet D et al., who is another colleague of Dick JE, used NOD/SCID mice to build a hu-leukemic-mice model.

The Road to Humanized (SCID) Mice for Acute Myeloid Leukemia

	Year	PMID	Journal, Author	Cell sources & used mouse strain
(1)	1979	292452	Br J Cancer, Palu G <i>et al</i>	AML cells into irradiated CBA mice
(2)	1984	6582788	Am J Pathol, Potter GK <i>et al</i>	AML cell line into nude mice
(3)	1991	1893976	Exp Hematol, De Lord C <i>et al</i>	AML cells into SCID
(4)	1993	8104540	Exp Hematol, Reiko Namikawa <i>et al</i>	AML patient BM cells into SCID
(5)	1994	7509044	Nature, Lapidot T <i>et al</i>	AML initiating cell into SCID
(6)	1997	9212098	Nat Med, Bonnet D <i>et al</i>	AML cells into NOD/SCID
(7)	1999	10477702	Blood, Ailes LE <i>et al</i>	AML progenitor cells into NOD/SCID
(8)	1999	16234360	Blood, Pearce DJ <i>et al</i>	AML cells into NOD/SCID
(9)	2002	12200698	Leukemia, Lumkul R <i>et al</i>	AML cells into NOD/SCID
(10)	2006	16998484	Nat Med, Jin L <i>et al</i>	AML cells targeting CD44 into NOD/SCID
(11)	2009	19570512	Cell stem cell, Jin L <i>et al</i>	Leukemic Stem cells w/CD123 monoclonal antibody into NOD/SCID
(12)	2005	17952057	Nat Biotechol, Ishikawa F <i>et al</i>	AML cells into NOD/SCID/IL2R γ _null
(13)	2007	21157036	J Clin Invest, Sarry JE <i>et al</i>	Leukemic Stem cells into NOD/SCID/IL2R γ _null
(14)	2014	25069538	Oncol Rep, Lee JY <i>et al</i>	LPB, BM-derived ALDH ^{dim} CD34 ⁺ CD38 ⁻ cells into NOD/SCID/IL2R γ _null

Fig. 2. Summary of hu-leukemic-mice model. To increase blast engraftment in BM, advanced mice model constantly has developed from nude mice model to NOD/SCID/IL2R γ _null mouse. Based on these, modified stem cell sources transplanted to optimize leukemia model.

Great benefits of NOD/SCID mice involve that successful engraftment of LSCs with high efficacy and low injected cells (Bonnet and Dick, 1997). Recently, most investigators prefer usage of NOD/SCID mice to investigate AML due to invariable probability in blast phenotype, morphology, and biologic properties (Ailles et al., 1999; Lumkul et al., 2002; Jin et al., 2006; Pearce et al., 2006; Jin et al., 2009). Nevertheless, incomplete NOD/SCID still exists including thymic lymphoma and high NK activity. Both mice, NOG mice by Ito M et al. and NSG mice by Shultz LD et al. displayed IL-2R γ^{null} , which abrogated the activation of NK cells (Ito et al., 2002; Shultz et al., 2005). Furthermore, AML cell infiltration in endosteal niche was firstly detected by Ishikawa F et al. using NOD/SCID/IL2R γ^{null} mice (Ishikawa et al., 2007). As part of these experiments, our group also reported that CD34 $^{+}$ CD38 $^{-}$ cells with high level of aldehyde dehydrogenase $^{\text{dim}}$ (ALDH $^{\text{dim}}$) positive cells is a reliable marker to make stable hu-leukemic-mice model for drug screening (Lee et al., 2014). Based on advanced model development without cessation, identification of LSCs and HSCs will be quickly revealed by modifying the hu-leukemic mice model. History of hu-mice model for leukemia study is briefly presented by Fig. 2.

Application and perspectives of hu-leukemic mice model

Regardless of the type of the cancer, the hu-mice model should be needed to investigate tumor microenvironmental explorer. Especially, interaction between immune cells and leukemic stem cells might be optimized through the hu-leukemic-mice model. Similar with hu-mice models, accomplishment of hu-leukemic-mice model for microenvironmental study gradually enlarged in their spectrum from *in vitro* study to *vivo* model (Fig. 3). Most leukemia accompanied by an impaired immune system leads to the failure of blast suppression. Thus, immune cell modulation as a therapeutic target is an emerging issue in treating AML. Inhibition of SIRP α in macrophage can eradicate AML stem cells (Theocharides et al., 2012) and VEGFR-3 antagonist treated NK cells can directly kill the leukemic blast. Because of the VEGFR-3 ligand, VEGF-C abundant status can affect

The Step for Humanized Mice Model

Fig. 3. Developments of hu-mice models. To mimic human condition, attempts have been developed from *in vitro/ex vivo* study to *in vivo* hu-mice models. The latest version of hu-mice model has a limitation to investigate relevance between microenvironmental factor such as immune cells and hematopoietic stem cells. To overcome this, notable protocol should be developed such as sequential transplantation, which first build hu-mice model with normal HSC, and then induce leukemia condition.

immune cell inactivation (Lee et al., 2014) and the feasibility of as a niche therapy such as immune cell and MSCs modulation is consistently presented by several papers (Lee et al., 2014; Cogle et al., 2015; Kim et al., 2015; Ayala et al., 2009). Also, another arguing point is about the hierarchical relevance between malignant niche and leukemic stem cells. Recently, Boyerinas B et al. showed that dormant leukemic cells are governed by niche, suggesting autonomous leukemic stem cell function as an authorizer in a leukemic microenvironment (Boyerinas et al., 2013). Conversely, Flach J insists that conversion of normal HSC into malignant cells occur by DNA damage, implying the importance of niche in the driving of malignant cells (Flach et al., 2014). To elucidate the controversy, an accurate interpretation could be possible through reproducible and credible *vivo* model, hu-leukemic-mice model. In addition, the progress of stem cell biology following the application of induced pluripotent

stem cells into the clinic and regenerative medicine will elevate the usability of the hu-mice model.

CONCLUSION

We briefly summarized the history of hu-mice model for immunology and pointed out the hu-disease mice model, especially for AML. Advanced hu-leukemic-mice models will contribute to analysis of machinery of underlying AML features and immunologic interaction. However, several concerns remain to be overcome, such as the standardization of individual variation in AML patients, successional expression of specific mutant genes including *FLT3-ITD* and *CBFB/MYH11* in secondary/tertiary transplantation. To completely treat AML, a better understanding of the immune niche and LSCs mechanism is needed and effective therapeutic protocols might be accomplished by mimicking the hu-mice system.

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Conflict of interests

The authors declare that they have no conflict of interests.

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