Concise Review

Patient-derived xenograft (PDX) models: characteristics and points to consider for the process of establishment

Etsuko Fujii[1], Atsuhiko Kato[1], and Masami Suzuki[1*]

[1] Research Division, Chugai Pharmaceutical Co., Ltd., 1-135 Komakado, Gotemba, Shizuoka 412-8513, Japan

Abstract: Tumor research has largely relied on xenograft models created by the engraftment of cultured cell lines derived from tumor tissues into immunodeficient mice for *in vivo* studies. Like *in vitro* models, such models retain the ability of tumor cells to continuously proliferate, so they have been used to predict the clinical relevance of studies on proliferating cells. However, these models are composed of a limited population of tumor cells, which include only those tumor cells that are able to adapt to culture conditions, and thus they do not reflect the diversity and heterogeneity of tumors. This, at least in part, explains the poor predictivity of non-clinical data in the research and development of molecularly targeted drugs. Recently, research focus has been directed towards patient-derived xenograft (PDX) models created by directly engrafting tumor tissues, which have not been cultured *in vitro*, into immunodeficient mice. PDX models reflect the diversity and heterogeneity of tumors, and the evidence they provide can be verified in the patient tissues from which they were derived originally. PDX models are anticipated to efficiently bridge non-clinical and clinical data in translational research. Based on the evidence obtained from our research experience, this review describes the characteristics of PDX models for acting as tumor models, and elucidates the points to consider when attempting to establish these models. (DOI: 10.1293/tox.2020-0007; J Toxicol Pathol 2020; 33: 153–160)

Key words: patient derived xenograft (PDX) model, engrafting tumor tissue, immunodeficient mouse, NOG mouse, EBV-related lymphoproliferative disorder

Tumor research has largely relied on xenograft models created by the engraftment of cultured cell lines derived from tumor tissues into immunodeficient mice for *in vivo* studies. Like *in vitro* models, such models retain the ability of tumor cells to continuously proliferate, so they have been used to predict the clinical relevance of studies on proliferating cells or of agents that exert anti-tumor effects by damaging and killing tumor cells, including DNA-damaging agents and agents that target driver mutations. But these models are composed of a limited population of tumor cells, only those able to adapt to culture conditions, and so do not reflect the diversity and heterogeneity of tumors. This, at least in part, explains the low predictivity of non-clinical data in the research and development of molecularly targeted drugs[1–3].

Recently, as a possible solution for this, there has been a focus on patient derived xenograft (PDX) models created by directly engrafting tumor tissues which were not cultured *in vitro* into immunodeficient mice[4, 5]. PDX models reflect the diversity and heterogeneity of tumors[6–8], and the evidence they provide can be verified in the patient tissues from which they were originally derived. Thus, the models are anticipated to efficiently bridge non-clinical and clinical data in translational research.

The development of immunodeficient mice has been a major contributor to the use of PDX models in medical research. The history of immunodeficient mice dates back to the 1960s when the athymic nude mouse was first discovered. In the following decades, efforts were made to improve the efficiency of establishing xenograft models. After the discovery and development of scid and NOD-*scid* mice, the NOD.Cg-*Prkdc^scid^ Il2rg^tm1Sug^*/Jic mice (NOG mice) and the NOD.Cg-*Prkdc^scid^Il2rg^tm1Wjl^*/SzJ mice (NSG mice) were developed as attempts to further improve the efficiency of xenotransplantation[9-12]. NOG and NSG mice lack mature T, B, and NK cells, and have multiple defects in dendritic cell and macrophage function. They are currently considered to be the animals best suited for engraftment of human tissues[4, 11].

Using evidence gathered from our research experience, in this review, we will describe the characteristics of PDX tumor models using NOG mice, and the points to consider when establishing such models.

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Characteristics of PDX Models

Our group has successfully established PDX models using NOG mice at PharmaLogicals Research Pte. Ltd., which is a joint venture founded by Chugai Pharmaceutical Co., Ltd., Mitsui & Co., Ltd., and the Central Institute for Experimental Animals. The establishment process of PDX models is outlined in Fig. 1. Surgically resected tumor tissues were engrafted subcutaneously into NOG mice, followed by three generations of serial transplantations and cryopreservation after the third generation. The cryopreserved tissues were thawed and transplanted into NOG mice for use as PDX models in various studies, including drug-response analyses and tumor biology research.

By engrafting several types of tumors from a variety of organs, we were able to establish epithelial and non-epithelial PDX models[13]. These models included epithelial tumors, such as, gastric, colorectal, and mammary cancers, as well as, non-epithelial tumors, such as, rhabdomyosarcoma, gastrointestinal stromal tumor (GIST), and astrocytoma. In addition to tumors from the original site, models were established from lymph nodes and other sites of metastasis. Similarly, successful engraftment of epithelial tumors including lung, mammary, and ovarian cancers, has been reported for the NSG mouse models[14, 15].

The established PDX models retained the same tissue structure, cell morphology, and differentiation found in PDX models of the original surgically excised tissues[13]. For example, in the three cases of colorectal adenocarcinoma with varying differentiation, the engrafted tissues that were serially transplanted for three generations successfully retained the pathological characteristics of the original tissues (Fig. 2). With well differentiated colorectal adenocarcinoma, ductal structures consisting of tall columnar tumor cells with slight atypia and clear polarization were retained. With moderately differentiated colorectal adenocarcinoma, ductal structures or fused cribriform structures consisting of atypical cells were retained. With poorly differentiated colorectal adenocarcinoma, there was no duct formation, and a growth pattern showing sheet or cord-like or single cell invasion of tumor cells into tumor stroma was well preserved.

Besides colorectal cancer, PDX models of other tumor types also successfully retained morphological characteristics. For example, with lung squamous cell carcinoma tissues, there was a tendency for differentiation from the basal side to the center of tumor nests, and a cornified layer or so-called cancer pearls were observed in the center of tumor nests (Fig. 3A). In renal clear cell carcinoma tissues, large clear cells formed sheet-like growths with a fine capillary network (Fig. 3A). With rhabdomyosarcoma, tumor cells with round nuclei with low cell adhesion proliferated in a diffuse manner, and some of these cells were racket-shaped, which is a noted feature of rhabdomyosarcoma (Fig. 3B). Engrafted GIST tissues consisted of tightly packed spindle cells (Fig. 3B).

In addition to the preservation of tissue structure and cell morphology, PDX models are known to retain the molecular biological and genetic features of the original tumor[4, 6, 7, 16–20]. Results of global gene expression analyses show that PDX models are designated to the same clusters as the original tumor, and that these PDX models retain key genetic and pathway activation[4, 20]. DNA copy numbers, genetic mutations, chromosome abnormalities and gene fusions are also retained in these models[19].

Next, we will take a look at interstitial cells, such as fibroblasts and endothelial cells. In primary engrafted tissues that were surgically removed from patients, cells originating from humans and mice were mixed together in the interstitial tissue[13, 21] (Fig. 4). By the third generation, there were no longer any human interstitial cells and the interstitial tissue constituted only of mouse cells[13]. Along with tumor cells, human cells were also maintained, whereas the interstitial cells were replaced by mouse cells during engraftment and passaging (Fig. 4). This is a defining characteristic of PDX models.

In PDX models, unlike *in vitro* cell-engrafted models, the tissue structure and cell morphology, along with their molecular biological and genetic features, are well preserved, and the models are anticipated to be applicable to various oncology studies. Multiple myeloma cells, which are difficult to maintain by passage *in vivo,* can be engrafted into NOG mice, and the NOG mouse models are known to preserve the characteristics of clinical tumors[22]. Thus, using NOG mice as hosts might enable the establishment of PDX models with tumor types that were difficult to establish in other immunodeficient mice.
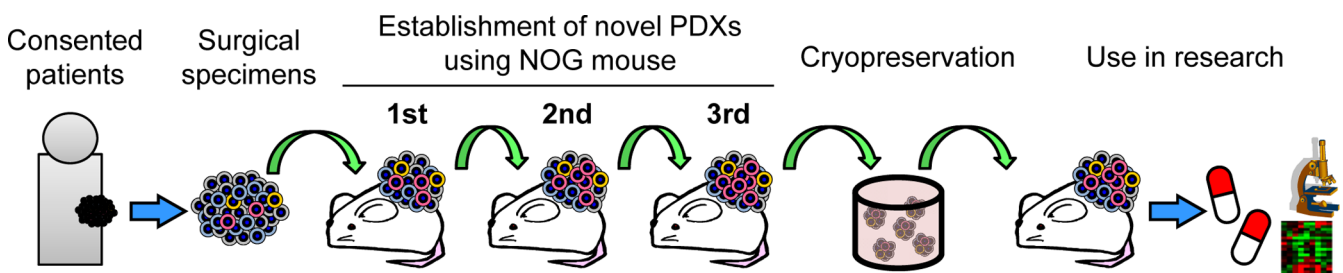

Fig. 1. Scheme for establishment process of patient-derived xenograft (PDX) models.

Points to Consider in Establishing PDX Models

PDX models are promising for oncology research, but there are also a number of obstacles for their successful establishment. Not all patient tissues can be successfully established as PDX models and PDX models of some tumor types are difficult to establish[1, 4]. Additionally, establishment of these models requires a lot of resources in terms of cost, time, and labor[1, 4]. Thus, in order to better utilize PDX models in oncology research, we should be able to establish them more efficiently for a wider variety of tumors.

In our previous study, the rate of establishment was 54/436 cases (16.6%), which was much lower than we had expected[13]. For various tumor types, the rates of establishment were approximately 30% for colorectal cancer, but less than 5% for breast cancer models, and there were no successful cases established for testicular and prostatic tumors. To address this, we analyzed all the causes of failure and categorized them into three types, namely, replacement of engrafted tissues by lymphoproliferative lesions (LPL), no tumor growth (NT), and attrition due to unscheduled death or host infections (DSI) (Fig. 5A). The causes differed with
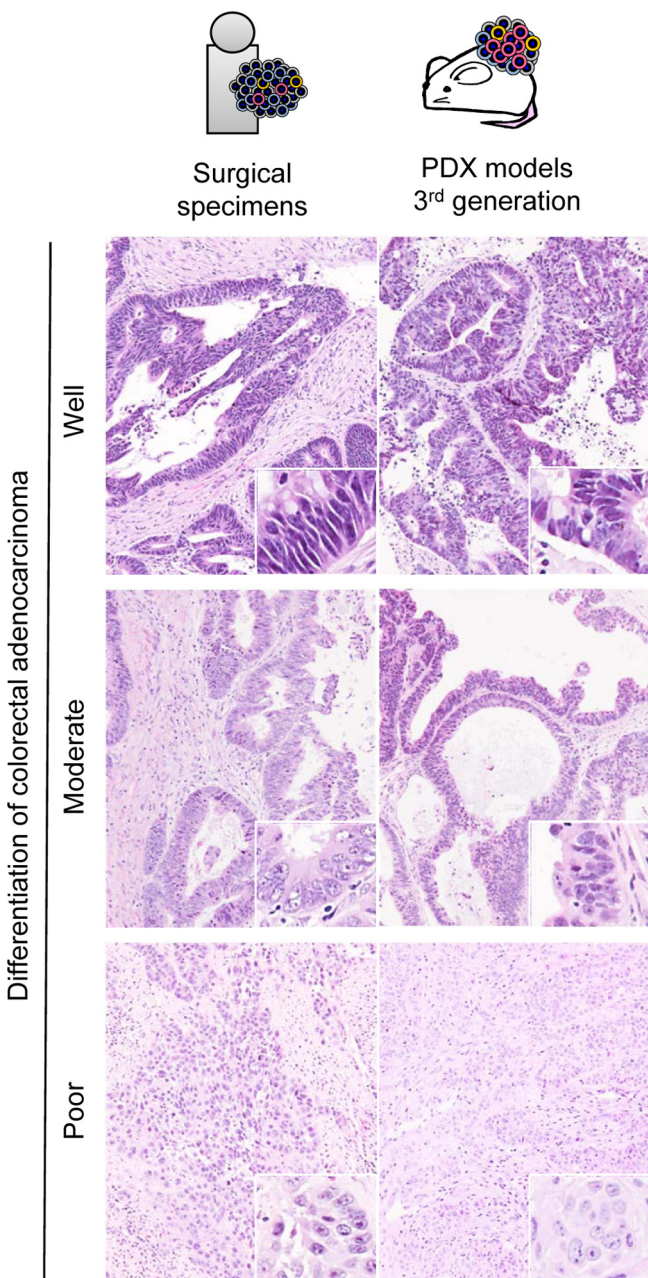
Fig. 2. Comparison of surgical specimens and transplanted tissues of colorectal cancer. Ductal structures are maintained with tall columnar cells in the well differentiated case and shorter columnar cells in the moderately differentiated case. Small nests of epithelial cells are formed in the poorly differentiated case.
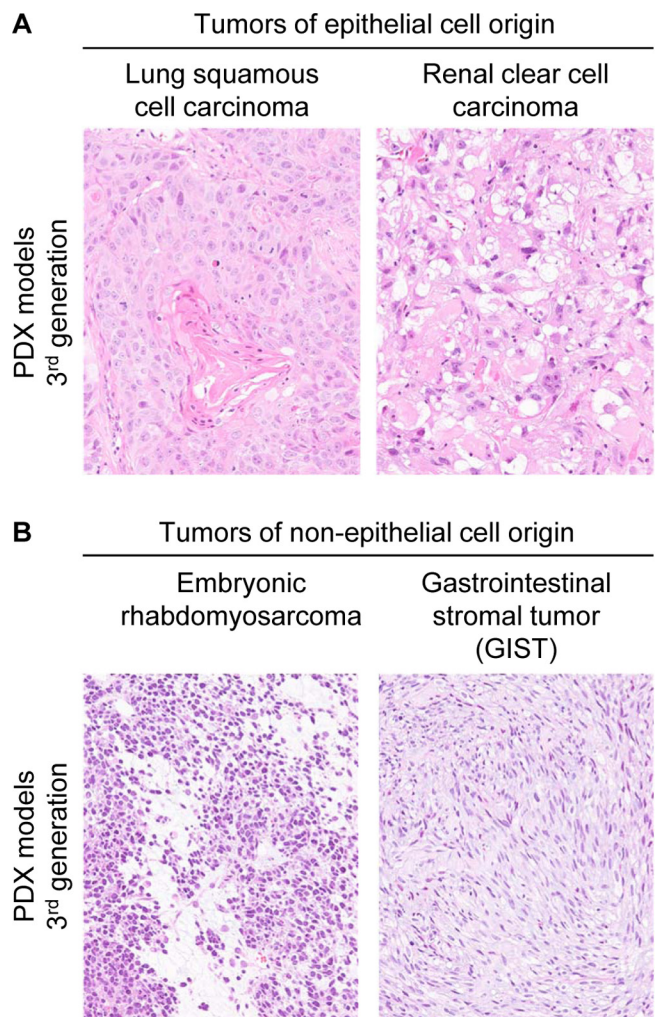
Fig. 3. Characteristics of representative epithelial and non-epithelial patient-derived xenograft (PDX) models established with the NOD.Cg-*Prkdc^scid Il2rg^tm1Sug*/Jic mouse (NOG mouse). A: Tumors of epithelial cell origin. 3rd generation tissues from a case of lung squamous cell carcinoma with characteristic cancer pearls, and renal clear cell carcinoma with sarcomatous features. B: Tumors of non-epithelial cell origin. 3rd generation tissues from a case of embryonic rhabdomyosarcoma with poorly adhering, round-shaped cells and occasional racquet shaped or myofibroblast-like features, and gastrointestinal stromal tumor (GIST) with an interlacing growth pattern of spindle cells.
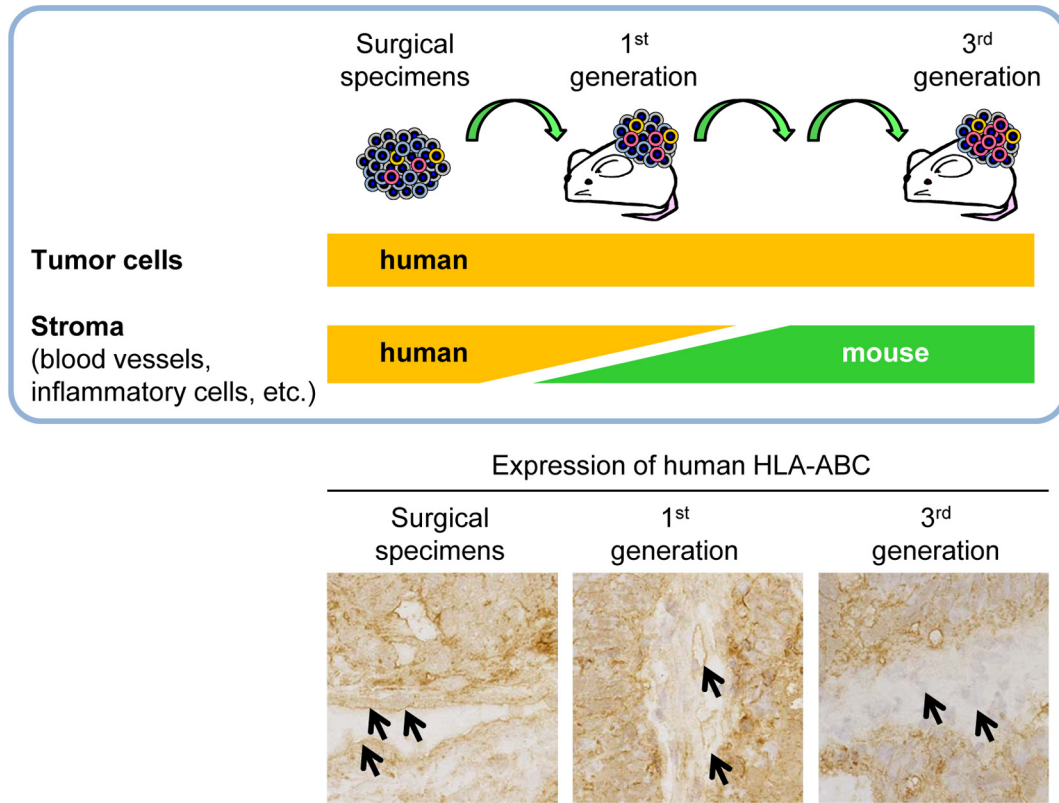
Fig. 4. Changes in the species origin of stromal cells during the establishment process in patient-derived xenograft (PDX) models. Expression of human HLA-ABC in surgical specimens and engrafted tissues of a case of colorectal cancer. In the primary engraftment tissue, there was a mixture of human and mouse stromal cells in the xenograft tissue. After 3 generations of passage, the stroma consisted of only mouse cells. Arrows show endothelium of the tumor interstitium.

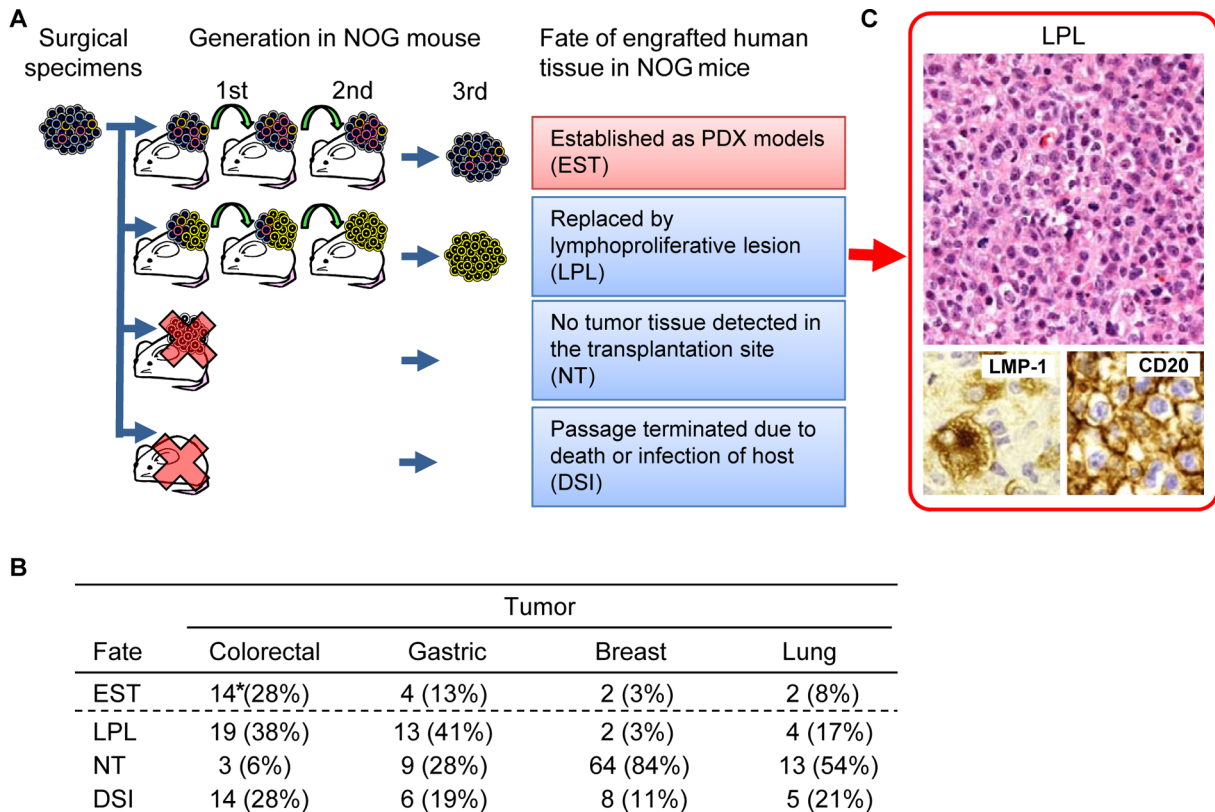| Fate	Tumor			
	Colorectal	Gastric	Breast	Lung
EST	14*(28%)	4 (13%)	2 (3%)	2 (8%)
LPL	19 (38%)	13 (41%)	2 (3%)	4 (17%)
NT	3 (6%)	9 (28%)	64 (84%)	13 (54%)
DSI	14 (28%)	6 (19%)	8 (11%)	5 (21%)

Fig. 5.

the tumor type, but the major causes for all types were determined to be LPL and NT[23] (Fig. 5B). With gastrointestinal tumors, LPL was observed in 40% of the engrafted cases, making it the leading cause of attrition.

In cases of LPL, Epstein-Barr virus (EBV)-infected B cells proliferate, resulting in the replacement of engrafted tissues with proliferating lymphocytes[23] (Fig. 5C). This change is thought to arise in EBV-infected B lymphocytes that are engrafted with the tumor tissue into the severely immunodeficient NOG mice, resulting in a state resembling lymphoproliferative disorders in human[24–27]. There are similar reports in NOD-scid and NSG mice engrafted with tumor tissues and 16–80% of the cases that were originally judged to be successfully established were in fact replaced by LPLs[28, 29]. LPL occurs often when tumor tissues are engrafted into immunodeficient mice, and hence it should be taken into consideration when attempting to establish a PDX model.

The infection rate of EBV in human is high—about 90% of the world population is infected with EBV[30]—so it is not feasible to eliminate EBV-infected cases for engraftment. Thus, we might improve the efficiency of establishing these models through early detection and attrition of affected cases during the establishment process. The most sensitive method for detecting LPL is to perform a thorough histopatholgical examination. However, this takes time, making it difficult to make a timely decision for all cases. Therefore, we recommend a scheme for deciding at necropsy. LPL is distributed in various organs of NOG mice (Fig. 6), and is frequently accompanied by splenomegaly (Fig. 7A). Based on this, we deemed that early detection and attrition could be achieved by monitoring gross findings at the time of passage[24] (Fig, 7B). Additionally, we have experimentally explored the possibility of prevention by applying an anti-CD20 antibody, rituximab, to eliminate EBV-infected lymphocytes[23].

With regards to the other major leading cause, NT, we analyzed various cases of colorectal cancer. We found that tumor-infiltrating lymphocytes (TIL) from the primary generation (first generation) mice reflect the immune contexture
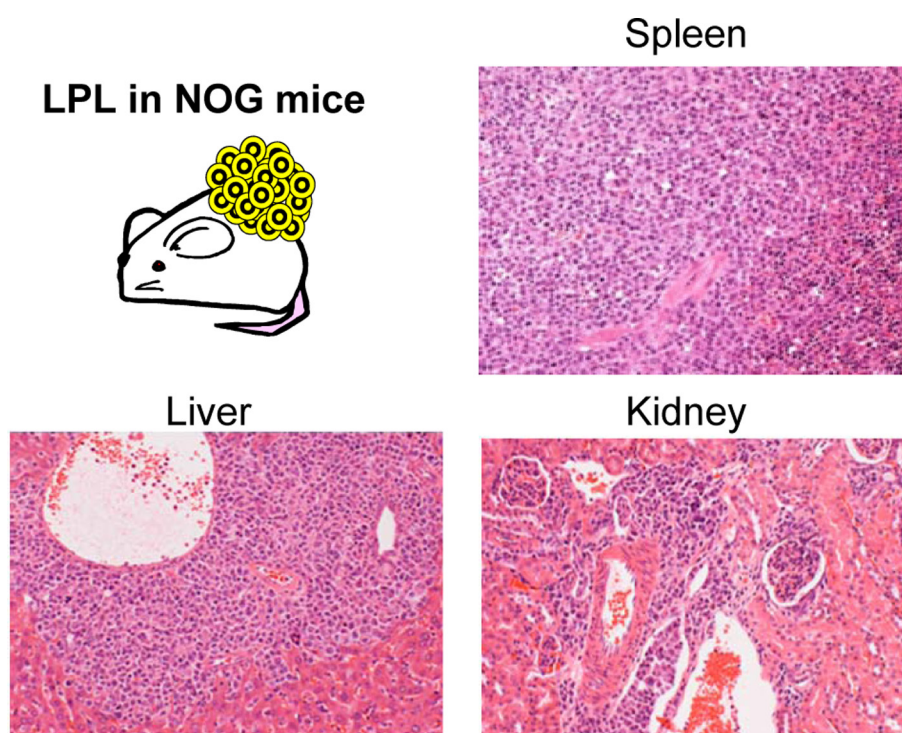

Fig. 6. Distribution of lymphoproliferative lesions (LPL) in NOD.Cg-*Prkdc*scid *Il2rg*tm1Sug/Jic mice (NOG mice). LPL is observed in the spleen, liver, and kidney.

Fig. 5. Fate of engrafted human tissues in NOD.Cg-*Prkdc*scid *Il2rg*tm1Sug/Jic mice (NOG mice). A: Analysis of the outcome of engrafted human tumor tissues. The tissues were passaged through 3 generations for establishment. In some cases, the palpable mass formed after engraftment consisted of a lymphoproliferative lesion (LPL) that was thought to replace the original tumor cells. In other cases, establishment was unsuccessful because no palpable mass was formed after engraftment or due to an unscheduled death of the mouse. B: Incidence of the fate of engrafted human tumor tissues. * number of cases. C: Analysis of LPL. LPL was characterized by examining leukocyte marker (CD20) and Epstein-Barr virus (EBV)-related antigen (LMP-1).
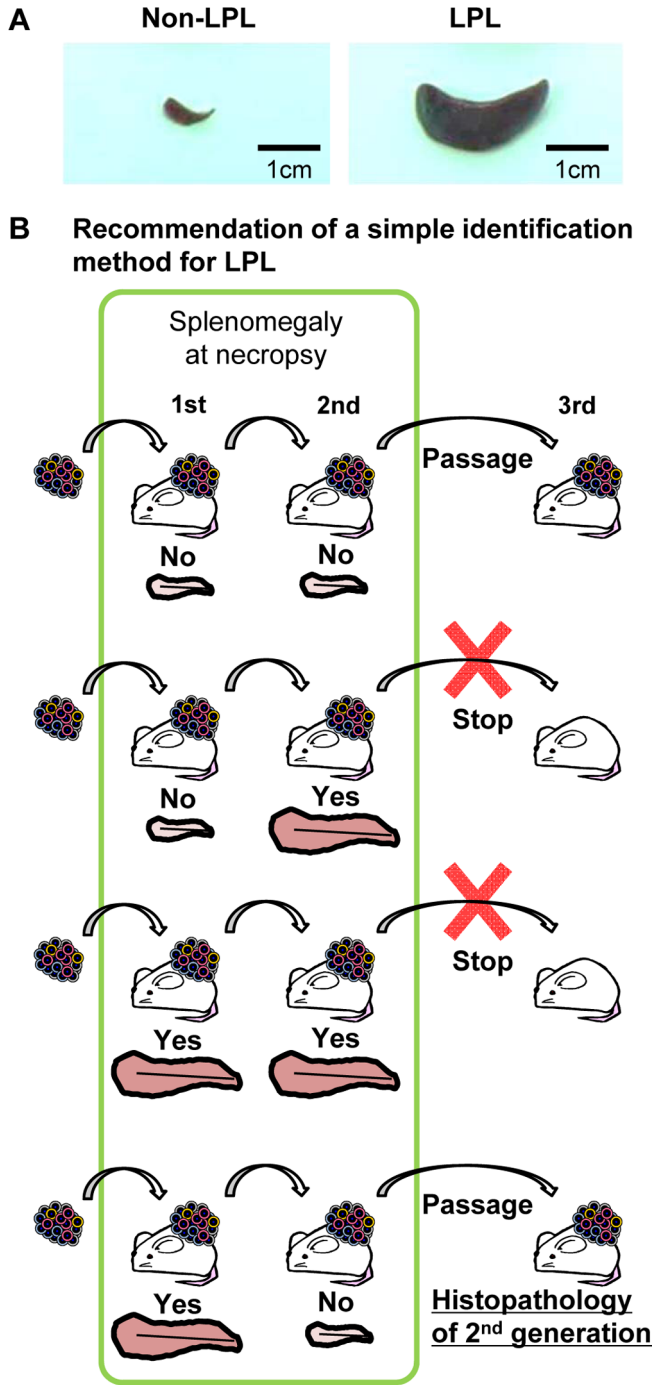
A

B Recommendation of a simple identification method for LPL

Fig. 7. Proposal of a method for early detection and early termination of lymphoproliferative lesions (LPL) cases at necropsy. A: Splenomegaly is observed in LPL-NOD.Cg-*Prkdc*^scid *Il2rg*^tm1Sug/Jic mice (NOG mice). B: The following decisions can be made at necropsy by gross examination of the mouse spleen: If there is no splenomegaly in the 1st and 2nd generations, passage should proceed, but if there is splenomegaly in the 2nd generation regardless of the results of the 1st generation, further passage should be terminated. If there is splenomegaly in the 1st generation but not in the 2nd generation, the tissue should be passaged, and the 2nd generation spleen should be examined histopathologically.

of excised patient tissue, and can suppress the progression and growth of tumor cells[31–34]. Thus, methods to manipulate the engrafted TIL, such as, elimination of lymphocytes and engrafting only tumor cells, or suppressing TIL by administering immunosuppressive agents to NOG mice might be effective in increasing the efficiency of engraftment.

With tumors that are affected by sex hormones, such as, breast or prostate cancer, the engraftment rate was notably low. There are reports of successful establishment of a breast cancer PDX panel that reflects the heterogeneity of the disease, by implantation into the mammary fat pad and subcutaneous administration of estrogen pellets [4, 20]. With prostate cancer, administration of testosterone is reported to improve the efficiency of engraftment. Hence, for hormone-dependent tumors, treatment with hormones is thought to be effective for improving the rate of engraftment. We have only studied the establishment process by sub-cutaneous engraftment, but orthotopic or sub-capsular engraftment into the renal capsule has been shown to be more efficient compared to sub-cutaneous engraftment, so considering the site of engraftment might also improve the rates of model establishment.

Future Perspectives of PDX Models

PDX models reflect the heterogeneity and diversity of clinical tumors, so they are utilized for studies in drug response or drug resistance mechanisms[5, 11, 35]. Especially for non-clinical research of molecular targeted drugs, the genetic characteristics of PDX models are verified, and PDX models with several specific types of genetic characteristics are selected to analyze drug responses[20]. Additionally, the models have been proven to be applicable for studying predictive biomarkers for drug responses and drug resistance. Because of these features, there is now a concept called co-clinical trials, which are studies conducted alongside clinical trials, and in such studies, PDX models are utilized to obtain data to support clinical trials[5, 20]. Furthermore, our research group has previously used PDX models for cancer stem cell research and we found that cancer stem cells transition between a proliferative and non-proliferative state according to the presence of drugs or other environmental factors[36]. In that study, we revealed a possibility that transition between states is one of the major features of cancer stem cells that contributes to resistance against cytotoxic agents and recurrence[36].

Thus, PDX models can be used to obtain novel evidence that could not be gained with *in vitro* cultured tumor cell models. While the types of tumors that can be established as PDX models are currently limited, the accumulation of further evidence towards stable and efficient establishment of PDX models might lead to expanding their use in oncology research.

Furthermore, there is active research being undertaken concerning regulation of tumor immunity, such as with immune check point molecules. For these studies, models

are constructed to reconstitute the human immune system through the engraftment of hematopoietic stem cells into severely immunodeficient animals, including NOG mice[37, 38]. The selection of appropriate hosts that mimic the human immune system, in combination with PDX models that reflect the heterogeneity and diversity of clinical tumors, might broaden the scope of novel research fields, such as tumor immunity.

Disclosure of Potential Conflicts of Interest: The authors declare that they have no competing interests.

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