Preclinical Animal Models of Cancer: Applications and Limitations

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Abstract

Despite the global advancement in availability of modern diagnostic tools and a variety of therapeutic modalities, many diseases have still been on the rise worldwide. Animal models serve as a valuable means in conducting preclinical research related to various human diseases including cancer. These models assist

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not only to understand the underlying genetic mechanism of a tumor but also the impact of several crucial genes that quite often gets mutated leading to deadly cancers. Scientists are striving for regulated and customized animal models which mimic the real cancers in human beings in their growth and development. For developing animal models of cancer, a variety of animals and personalized as well as precision medicine methods are being used to cater the demand of different patients. Of these animal models of cancer, several mice models have been used worldwide due to their uniqueness in mimicking human cancers. Here, we have discussed about the major benefits and constraints related with the use of such models.

Introduction

Uncontrolled growth of cells, i.e., tumor, has become one of the major diseases that fatally endanger an individual's health, as a result of proficient control of most important infection causing diseases and the extension of quality and disease-free human life. As per a 2015 World Health Organization (WHO) report, tumor formation is one of the main causes of death among individuals aged between 70 and 91 years in the developed countries (Bray et al. 2018). The number of new cancer cases per year is predicted to rise from 18.1 million in 2018 to 29.4 million in 2040 as an outcome of combined effects of growing and aging population (Wild 2019). Cancer has already become a serious problem in both developing and underdeveloped countries because of delayed detection of most cancers and inadequate prevention measures. To overcome this global burden, highly innovative approaches and new diagnostic techniques are required. Experimentation with animals and/or animal models acts as a bridge to fill the gap between cell culture (in vitro) experiments and clinical research. Animal diseases have analogous occurrence and development to human diseases under specific situations. Further, animal models encompass similar genetics, physiology, and anatomy to humans. Therefore, animal models are helpful to conduct research related to several human diseases including cancer. Animal models can aid in understanding the genetic foundation of a tumor and the impact of certain genes and gene mutations in the initiation and promotion/progression of tumor besides designing and testing of anticancer medicines (Schachtschneider et al. 2017). Scientists are striving for regulated and customized animal models that are more close to human cancers since personalized and precision medicine continues to advance (Xu et al. 2019). To develop animal models of cancer, a variety of methodologies have been used in several animals. Additionally in tumor studies, every tumor mice model has its own peculiarities.

Mouse Model

Prior to the expansion of animal genetic models, researchers have employed cell culture systems using cell lines obtained from human malignancies to study cancer, though use of such methodologies to obtain the useful details that are vital for cancer research has certain limitations too, such as inability to analyze physiological interactions between tumor cells and their environment in vivo. Some of these constraints can be overcome by using xenograft models where human tumor-derived cell lines are transplanted in mice (Cheon and Orsulic 2011). Most of these investigations must be conducted using subcutaneous implantation and immune-compromised mice to avoid immune responses and site-specific interactions. For both morphologic and genetic levels, tumors can be quickly generated favoring human tumor cells within a perfect mouse system. Several mice models have provided an invaluable tool for investigating tumor initiation, promotion/progression, and treatment response. Additionally, cancer biologists now have access to a variety of genetic manipulation tools (Walrath et al. 2010). Choosing the right technique for creating mice models of cancer has been a crucial initial step in any such research study. Furthermore, determining the deliberate aims of individual mouse models is also crucial.

Mice are considered to be the most valuable research animal and perhaps the most profitable vertebrate in the contemporary times due to the fact that they offer various advantages including individual cages that can hold multiple animals in a little area; they have a short life cycle as tiny as 9 weeks among generations for specific strains, also being prolific breeders. For mice, a huge range of tools and reagents are available, allowing researchers to investigate practically any component of the immune response. Additionally, the genomes of human beings and mice species have many similarities, such as amount of genes coding for the proteins (Baxter and Griffin 2016). The murine histocompatibility complex has been well understood, and many features of humoral, innate, and cellular immune responses were first reported in mice and later found in humans. Because of these qualities, the mouse has become the favored animal for studying the host immune response to a variety of virus infections. Despite significant progress in understanding the underlying causes of uncontrolled growth of cells and the identification of anticancer medicines in recent years, effective clinical translation of these techniques remains a challenge (Landgraf et al. 2018).

Humanized Mouse Model

The key reasons state that the majority of cancer research uses rodent models, which differ physiologically from humans in a variety of ways. As a result, rodent cancer models are unable to exactly mimic the constitution of cancer patients, necessitating the evolution of new cancer models that are superiorly suited to broadly signifying the compound features of human tumors, thereby allowing for betterment of central

and translational research (Perrin 2014). The first animals with malignancies of human origin created using this method were the nude mice with T-cell-deficient and immunized with human cancer cell lines, also known as cell-derived (CDX) models. These rodent models have become a common in vivo stage for studying oncogenesis related to human body and assessing the efficiency of anticancer drugs (Fogh et al. 1977). Furthermore, the evolution of mice strains with more severe immunodeficiency such as NOD/SCID mice and NOD/SCID IL2rg/mice made it easier to use rodents to competently repopulate primary human tumor cells and mirror the diverse features of these cancers in patients [patient-derived xenograft models (PDX models)] (Hidalgo et al. 2014).

In tumor studies, both human oncology investigations and anticancer medication intervention are greatly aided by the use of CDX and PDX models. Current studies have revealed that the lack of human-resistant components in these types of animals may critically limit their utility in advanced studies and the development of innovative human cancer therapies (Aparicio et al. 2015). Humanized mice are genetically modified to carry genes, defected cells, and tissues, allowing them to mimic human traits (Shultz et al. 2007). HIS mice (humanized mice with a functional immune system of humans) could be a useful animal to study the relations among human immunological mechanisms and tumors as well as helpful in the development of antitumor interventions (Hu and Yang 2012).

Immunodeficient Mouse Model

Human tumor cell engraftment in immunocompetent models is hampered by robust xenogeneic immune rejection (Yang and Sykes 2007). Various mouse strains which are enabled to produce sufficient immune responses have been created by interrupting key genes involved in resistant cell formation, endurance, and functionality. The capability to create these animals which are able to produce sufficient immune responses is essential for creating humanized mice that can be used to study human cancer progression. Immunodeficient mice were produced indeed to overcome the rejection of human cancer cells by the mouse adaptive (T and B cells) and innate (NK cells and macrophages) immune systems. For instance, forkhead box N1 (*Foxn1*) and protein kinase, DNA-activated, catalytic polypeptide (*Prkdc*) gene (Bosma et al. 1983) deletion causes T- or B-cell deficiency in mice; deletion of interleukin 2 receptor subunit gamma (*IL2rg*) (Ito et al. 2002) or β2-microglobulin (*B2m*) genes leads to the absence or functional impairment of mouse NK cells, recombination-activating gene 1 (*Rag1*) (Mombaerts et al. 1992), and recombination-activating gene 2 (*Rag2*) (Shinkai 1992), although selection of nonobese diabetic (*NOD*) or *NOD Sirpa* genes prevents phagocytosis by mouse macrophages (Table 1). Combinations of these genetic engineering strategies have been applied to develop the popular immunodeficient mouse strains such as NOD/Prkdcscid (NOD/SCID), NOD/SCID IL2rg−/− (NSG or NOG), and Balb/c Rag1−/− IL2rg−/− (BRG) that have all been used in human cancer research.

Single-gene mutation models such as nude mice (*nu*) strains and severe combined immunodeficiency (SCID) strains, nonobese diabetic strains, *RAG* (recombination-activating gene) strains with targeted gene deletion, and a variety of hybrids derived

Table 1 Various gene deletions and their effects on mice

S. No.	Deleted gene	Effect of deleted gene on mice	Reference
1.	Forkhead box N1 (*Foxn1*), recombination-activating gene 1 (*Rag1*), recombination-activating gene 2 (*Rag2*), and protein kinase, DNA-activated, catalytic polypeptide (*Prkdc*) gene	T- and B-cell deficiency in mice	Bosma et al. (1983); Mombaerts et al. (1992); Shinkai (1992)
2.	Interleukin 2 receptor subunit gamma (*IL2rg*), β2-microglobulin (*B2m*) gene	Functional impairment of mouse NK cells	Ito et al. (2002)
3.	Nonobese diabetic (*NOD*) or *NOD Sirpa* genes	Prevents phagocytosis by mouse macrophages	Ito et al. (2002)

from crossing double and triple mutation mice strains with additional defects in innate as well as adaptive immunity are among the immunodeficiency mouse models.

Patient-Derived Tumor Xenograft Models

Human xenograft models, which involve human cell lines transplanted into hosts with weakened immune systems such as SCID mice, are widely used models for evaluating cancer cell killing medicines. These models are very useful for the development of chimeric antigen receptor (CAR) medicines, which can grow xenografts for antitumor efficacy testing using either human cell lines or patient-derived materials (Siegler and Wang 2018).

The degree of immunodeficiency of the murine host is one of the essential parameters that determine the efficacy of human xenograft models for various immune system-enhancing uses. T-cell function is impaired in athymic nude mice because they lack normal thymic development. Many parts of the immune response, however disrupted, are present in athymic nude mice because functioning innate immune populations such as neutrophils and dendritic cells, as well as B cells and natural killer (NK) cells. As a result, in this model, engraftment of human hematopoietic elements and other primary human cells is extremely limited. SCID mice lack a DNA-dependent protein kinase that is necessary for T- and B-cell development, while Rag-deficient mice lack the *Rag1* and *Rag2* genes, which are also required for T- and B-cell function. The ablation of the *IL2r* chain results in concurrent impairments in the functions of the IL2, IL4, IL7, IL9, IL15, and IL21 receptors, as well as mice lacking NK cells. The immunodeficiency in the ensuing mice worsens as a result of combining genetic defects, and the engraftment of donor human immune cells improves as a result. Thus, mice produced from SCID, Rag1null, or Rag2null animals with a specific mutation in the *IL2r* gene are the best for engraftment of human hematopoietic stem cells (Hasgur et al. 2016).

The NOD/SCID *IL2r* chain knockout mouse, preclinical model with engineered combined immunodeficiency has been frequently used hosts for chimeric human-mouse immune reconstitution and various tissue chimaeras (Walsh et al. 2017). Although athymic nude mice were adequate for engraftment of human cancer cell lines, NSG mice and their counterparts are necessary for engraftment of real human tumors (Puchalapalli et al. 2016). These primary tumor samples were used to create PDXs that accurately model the complexity involved in natural tumor development, including genomic heterogeneity, tumor architecture, and microenvironmental factors, which is critical for developing an effective in vivo preclinical tumor model for therapeutic evaluation (Byrne et al. 2017).

Given the importance of immunotherapy in various human cancers and the disadvantages of both identical gene cell line-based models and GEMMs in producing tumors that accurately reflect the genetic and physiologic heterogeneity of human cancers, there has been a growing interest in developing methods of human immune reconstitution within PDX models in order to create an experimental humanized model for evaluation of immunotherapy. The regenerated human hematopoietic system and tumor from the same patient must theoretically match in this experimental setup. There are hurdles to the effective development of humanized PDXs.

For successful tumor proliferation, among several mice in a realistic timeframe, a PDX model requires high take rates. The hematopoietic system, unlike the PDX models, cannot be formed from a single tumor that is transmitted through several mice; therefore recurrent persistent sampling would be required to form individual humanized PDX mice.

Murine Tumor Mouse Models

Genetically Engineered Mouse Models

Our understanding of the hereditary basis for tumor has advanced dramatically in the last two decades, followed by the advancement in genetic engineering technologies. These advancements result in the development of mice that incorporate precise genetic changes to allow tissue-specific autochthonous tumor growth. These models primarily use tissue-specific promoters to induce tumor gene expression via either SV 40 vector or genes pertinent to tumor formation (Greenberg et al. 1995), such as tissue-specific recombinase enzyme expression to drive tumor suppressor gene deletion (such as *PTEN* and *TP53* in prostate cancer (Chen et al. 2005) or *APC* in colon cancer (Shibata et al. 1997), *Kras* and *MYC* in breast cancer (Sinn et al. 1987), and *BRAF* V600E in melanoma (Hooijkaas 2012)).

In prostate cancer models, these genetic changes can be used to drive autochthonous invasive development of cancer and to create precancerous lesions such as prostatic intraepithelial neoplasia or pancreatic intraepithelial neoplasia (*PanIN*) (Kaplan-Lefko et al. 2003) in pancreatic cancer models (Hingorani et al. 2005). For immunotherapeutic intervention, extensive windows are required to establish an efficient anticancer immune response and simulate immune-associated side effects that are made possible by the longer period of tumor formation and progression.

This prolonged genesis and spread of tumor allow the autochthonous establishment of a compound tumor microenvironment, as genetically designed models stimulate neoplastic transformation of healthy cells at the necessary organ location to drive tumor development. In comparison to syngeneic tumor models, this significant advantage of GEMMs makes them particularly useful for assessing immunotherapeutic modalities. The quantity and composition of immune cell infiltration are both governed by native immunosuppressive stroma and vasculature, both of which are present in the novel tumor microenvironments that develop in the setting of GEMMs. Models can also be utilized in which the alteration driving tumor development is related to the tumor immune microenvironment, which can impact immunotherapy effectiveness like *PTEN* loss which has been linked to an immunosuppressive tumor microenvironment in melanoma, making it possible to test therapeutic modalities making these malignancies more sensitive to immunotherapeutic intervention using models in which *PTEN* loss is the driving force behind tumor formation. This is in dissimilarity to human disease which is most commonly affected by the steady accrual of mutations in a lesser fraction of cells contained by the organ of origin, eventually leading to transformation (Peng et al. 2015).

GEMMs which exploit tissue-specific promoters stimulate development of cancer in entire cells of that lineage. Furthermore, the mutational load in genetically engineered models may not be equal to that described in the corresponding human illness due to overexpression or deletion of a specific number of genes. Increased mutational load and subsequent neoepitope production are a significant issue for evaluating the efficacy of ICB, so this is critical when evaluating immunotherapies (Goodman et al. 2017). It is conceivable to increase carcinogenesis while also driving the accumulation of new mutations by targeting genes linked with mismatch repair and genomic stability [such as *MLH1* (Germano et al. 2017), *BRCA1/2* (White et al. 2008), *APC* (Shibata et al. 1997), and *mTERT* (Bojovic and Crowe 2011)]. This enhanced mutational rate encourages the production of novel antigens that CD8+ T cells can detect, thereby making immunoediting easier (Yarchoan et al. 2017). Moreover, increasing genomic instability may enhance the coevolution of the anticancer immune response and tumor escape pathways, potentially leading to immunotherapy confrontation.

Challenges Faced by GEMMs

However, GEMMs have significant merits in the evaluation of immunotherapeutics; they face some of the logistical obstacles that prevent them from being used in the evaluation of cytotoxic drugs.

1. A major challenge when employing GEMMs is, consider the entrance of the model tumor phenotype and neoplastic development latency depending on the mechanisms, this can vary dramatically and be used to promote the growth of tumors (Ku et al. 2017). The development of GEMMs that target numerous tumor suppressors that can boost penetrance and decrease latency can help to overcome some of these limitations.

2. To monitor tumor progression, standardize treatment scheduling, and track the kinetics of anticancer immune responses, noninvasive imaging techniques such as ultrasonography or magnetic resonance imaging (MRI) are required.

3. It is vital to assess whether the murine immune target is cross-reactive with the comparable human target, just as it is with syngeneic models. This comprises antigens and surface markers found on human immune cells as well as malignancies not found in mouse cells.

4. Cross-reactivity is especially important in the development of immunotherapeutic vaccines that detect antigens in the context of human MHC class I in patients; GEMMs that integrate human MHC class I and MHC class II have been designed to analyze peptide-specific T-cell responses that are relevant to human antitumor immune responses in order to examine these peptide-specific responses (Pajot et al. 2004). Furthermore, expression models that incorporate target antigen expression into GEMMs (DuPage et al. 2011) have been established enabling the quick construction of models that may be used to test antigen-specific immunotherapies. Antigen processing differences occur between mouse and human antigen-presenting cells, affecting cross-reactivity with human epitopes and underlining the fundamental issues that afflict all preclinical models that employ murine vs human cancer cells. As a result, the best paradigm for evaluating immunotherapies would be responses to human cancers in immunocompetent models.

Tumor Cell Lines with Identical Genes

Tumor models with similar genes are the most well-known and widely used in preclinical trials for evaluating antitumor drugs. It is feasible to obtain impulsive, mutagen-induced, or recombinant cancer cell lines from inherent strains such as C57BL/6, BALB/c, and FVB mice which can then be extended in vitro and utilized to immunize wild-type hosts to generate a cancer-bearing system. These animals are mainly valuable in the evaluation of immunotherapy medicines because they can be used to evaluate the formation of new antitumor immune responses without the need for adoptive immune population transfer. The most noticeable applications of syngeneic tumor models are the use of mutagens to induce tumor growth in mice and then testing the anticancer efficacy of tumor immunotherapies in these tumor-bearing mice (Schreiber et al. 2011). These models were employed by Schreiber et al., to detect and characterize the process of transformation of normal cells into clinically detectable tumors using chemicals such as methylcholanthrene (MCA). The impact of mutagen-induced cell lines on tumor formation and antitumor resistant responses as well as the evaluation of immunotherapy can all be investigated (Uno et al. 2006). Carcinogen-induced cancer models, in contrast to genetically specified tumor models, have a higher level of gene instability, ensuing in the formation of a more "physiologically realistic" tumor microenvironment. However, this complexity comes with its own set of obstacles, such as penetrance of tumor, issues related to latency, and lack of shared tumor allergens.

Many of these mutagen-derived tumors were used to create cancer models which are frequently employed to create mouse tumor models with identical genes. The

most vital advantages of tumor cell lines with identical genes is their simplicity. Because they use tumor cell lines that can be rapidly and reproducibly expanded in large numbers prior to implantation into hosts, these syngeneic models can be used for studies that require large group numbers that are difficult to obtain using genetically engineered models or patient-derived xenografts (PDXs). Another advantage of using syngeneic tumor models is that they may be genetically manipulated to evaluate particular tumor cell-intrinsic immunotherapy sensitivity or resistance biomarkers. In research assessing antigen-specific vaccination methods, tumor cells engineered to express the target antigen, for example, can be used to investigate anticancer effector responses in vitro and in vivo. It can be challenging to target antigens whose expression is typically limited to organs for which there are no suitable preclinical models. It is also possible to weigh the relative relevance of a variety of factors that might affect immunotherapeutic effectiveness. This is especially true in checkpoint blockade, where checkpoint ligands may be removed or altered to determine their function in the anticancer response.

Challenges Faced by Tumor Models with Identical Genes

Tumor models with identical genes have turned out to be the most widely used preclinical model for the assessment of immunotherapy due to their ease of use and experimental reproducibility. However, these practical advantages also highlight one of the system's drawbacks. The genetic and microenvironmental heterogeneity that characterizes cancer is missing from these models. Tumor heterogeneity causes both intrapatient and interpatient heterogeneity, which makes each patient's malignancy distinct (Hanahan and Weinberg 2011). This is the most thought-provoking elements of creating effective cancer treatments; therefore an ideal preclinical system for researching immunotherapeutics would also correctly represent this diversity. Syngeneic tumor models, on the other hand, are woefully deficient in both areas. The cell lines are transplanted into a small number of inbred mouse strains that lack interpatient variability and lack mutational patterns that mirror human intrapatient genomic heterogeneity. The absence of cancer stem cells and other progenitor populations in the tumor microenvironment contributes to the lack of mutational heterogeneity in syngeneic xenograft tumors. This might be a long-term source of tumor mutational evolution (Shackleton et al. 2009). Furthermore, mutational heterogeneity requires clonal development of differentiated cancer cells, which may be problematic in many syngeneic murine models due to their lower levels of genomic instability than humans (Prowse and Greider 1995).

In addition, tumor models with identical genes have typically experienced considerable selection as a result of adaptation to severe in vitro or in vivo conditions, resulting in clonal diversity limitations. In vivo implanted syngeneic tumor cells can behave differently throughout tumor growth. Numerous lineages can be introduced into tumors to overcome these barriers, leading in tumors with multiple populations (Calbo et al. 2011).

During the immunoediting process, this artificial heterogeneity lacks the tumor cell-intrinsic functional flexibility that permits tumors to continually adapt and develop in response to the immune response. Another problem with animal models

with identical genes is that the implanted tumors develop as new poorly differentiated malignancies rather than going through the natural stages of tumor evolution that genetically modified models do like premalignant transformation, tumor development, and progression (Greenberg et al. 1995).

In most syngeneic tumor models, this causes tumor growth to be sped up, resulting in tumor expansion over a period of weeks. Because anticancer immune responses produced by immunotherapy normally have a latency period prior to growth and maturation, therapeutic advantage is often detected as improvement in overall survival rather than objective clinical reactions (Madan et al. 2010). As a result, the rapid kinetics of tumor growth in syngeneic models frequently provides an insufficient time frame for assessing immunotherapy efficacy. Furthermore, it prohibits the study of immunotherapeutics at initial stages of disease, which has been indicated as a possible best time to begin interventions by activating the immune system in some abnormal cells in order to maximize clinical benefit (Gulley and Drake 2011).

Zebrafish (*Danio rerio*)

Over the years, the zebrafish (*Danio rerio*) has become a popular and widely used animal model in preclinical cancer research. The zebrafish is a tropical freshwater fish belonging to the Cyprinidae family and the Actinopterygii class that lives primarily in the Ganga river in India.

The fish was initially utilized as a study replica in the year 1970 by a researcher George Streisinger, who chose it over the mouse model because of its simplicity and ease of genetic manipulation. However, in the 1990s, the usage of zebrafish as an animal model grew, as two scientists exploited the model to create two huge mutant lines (Dahm et al. 2006). Zebrafish and its capacity to faithfully mimic a range of human malignancies make them a valuable in vivo system for drug discovery and validation.

Transgenesis, gene inactivation, transplanting, and carcinogenic induction all have been used to create zebrafish models of human cancer that have shown to be molecularly and pathologically comparable to their human counterparts. The suppression of cancer-relevant traits allows researchers to find and test effective drugs in embryonic and adult zebrafish. Following the selection of suitable compounds, preclinical testing in mammalian models can be carried out, allowing lead compounds to enter human trials quickly and efficiently. From past decades, zebrafish has been utilized as a scientific animal. Because of the obvious advantages of zebrafish, including large clutch sizes, transparent embryos, and embryo development outside of the womb, the initial focus was on developmental biology. Studies have indicated that zebrafish may form practically every type of tumor on their own. There are various most prevalent targets for spontaneous neoplasia: testis, gut, thyroid, liver, peripheral nerve, connective tissue, and ultimobranchial gland. Furthermore, the zebrafish model provides a number of advantages for therapeutic nanoscale drug delivery systems, particularly in terms of optical transparency. Its

zebrafish embryo's transparency allows researchers to see within the fish body in real time, such as organ or tumor development and vascular expansion. The embryos of zebrafish remain translucent until 60 hours after fertilization (hpf), when the coloring process begins. To maintain their transparency beyond that, the embryos are treated with chemical called 1-phenyl 2-thiourea (PTU), which inhibits the coloring process, or transgenic mice like Casper, which lack pigments on their skin, can be utilized instead (McGarth 2008).

The existence of replicated factors resulting from a recent partial or total genome replication in teleosts is a recurring problem in zebrafish, which may alter the role of tumor genes and tumor inhibitors in tumor genesis. For example, in the zebrafish genome, there are two variants of pten that are functionally superfluous in growth but not in tumor genesis. There will be no defeat of pten a as found in the defected cells of homozygous pten b mutants (Feitsma and Cuppen 2008). Because zebrafish is an excellent model for molecular investigations of many human diseases, cancer is one of the most studied disorders utilizing this model. This is owing to the models and its ease of design, which includes genetic editing, xenotransplantation, and chemical exposures. In zebrafish, for example, overexpression of the *Myc* gene causes T-cell leukemia, exposure to dimethylbenzanthracene causes intestinal cancer, and transplantation of human cancer cells such as B16-F10 melanoma cells causes melanoma in the model (Harfouche et al. 2009).

Pig (*Sus scrofa*)

Animals have extensively been used to learn more about human abnormalities, and they continue to play an important role in cancer research (Wright et al. 2008). Nonmammalian creatures like zebrafish can provide useful information. Zebrafish have the unique benefit of having naturally transparent embryos and larvae, as well as the ability to produce transparent adults (Antinucci and Hindges 2016). This makes it easier to research and track tumor vasculature (Nicoli et al. 2007), spread of cancer cells to other body organ (Wang et al. 2014; Liu et al. 2017), and anti-vasculature (Jing et al. 2018; Landgraf et al. 2018) drug evaluation in vivo. Zebrafish, on the other hand, are extremely different from human in terms of mass, overall survival, and most importantly, environmental influences. Bigger models such as dogs, cats, nonhuman primates, and *Sus scrofa* have been used as research species because they have some parallels to humans. Although dogs and cats grow cancers on their own (MacEwen 1990; Alvarez 2014) and their veterinarian action has supplied precious source for tumor studies, the general public does not accept the utilization of attendant models in properly scientific investigation. Nonhuman primates are also restricted due to tight restrictions imposed and ethical considerations. Pigs, on the other hand, have been domesticated as a source of food for generations. Their human and legally used animals as experimental models under controlled situation raise less problems (Perleberg et al. 2018). *Sus scrofa* are quite suitable animals for biomedical study because they split numerous parallels with human in

terms of mass, size of body organs, structural design, physiology, and pathology (Lunney 2007).

Pigs have traditionally been used to investigate the effects of nourishment, to evaluate new surgical events or advance organ transplant, and to create imaging technologies that may be employed on a human scale in addition to their reasonably long longevity of 12 to 15 years (Hoffe and Holahan 2019). Long-term research can be conducted to evaluate or verify new biological markers, treatments, or imaging choice and track illness development and failure in a single model (Flisikowska et al. 2016). Pigs have identical pharmacokinetic reactions to human beings in pharmacological studies (Myers et al. 2010). Pigs have thus been identified as a valuable animal model for translational drug discovery.

Genetically Modified Pigs

Palmiter and Brinster were the first to use DNA microinjection into the pronuclei of fertilized oocytes to make transgenic rabbits, sheep, and pigs (Hammer et al. 1985). However, only 1–5% of transgenic progeny were produced as a result of this approach (Tian et al. 2018). Porcine oocytes have high lipid content, making it difficult to see the pronuclei. The production of a large number of nongenetically modified models frequently larger than 95% was both ethical and practical. Furthermore, as initially envisioned, DNA microinjection only allows for the inclusion of transgenic genes at random places in the host genetic material. As a result, more competent and adaptable techniques were sought.

Transgenic pigs expressing a *Cas9* transgene that is either inserted at the ROSA26 locus in a Cre-dependent manner (Wang et al. 2017) or universally all through the body have recently been created, inspired by work done in mice (Platt et al. 2014). By delivering single or many guide RNAs with or without Cre-recombinase to somatic cells in vivo, gene editing of somatic cells can be done in vivo. Although in vivo genome editing is still in its infancy, it promises to be a potent tool for modifying the genome in specific organs and cell types at any age. Tumor-supportive genes, for example, can be tweaked in multiple rounds of gene editing to replicate the accumulation of changes that occur as tumor entities move forward (Makohon-Moore and Iacobuzio-Donahue 2016). Somatic modification also allows researchers to study mutations that would otherwise be deadly or detrimental in specific tissues or organs without having to worry about their consequences on the rest of the body. In practice, Cas9-expressing animals can shorten the time it takes to generate and breed new lines with desired mutations, which is a considerable benefit for bigger species.

Porcine Cancer Models

Although the study of porcine cancer biology is still in its early stages, preliminary evidence suggests that pigs can accurately mirror human tumors. In wild-type pigs, spontaneous malignancies develop infrequently, and in humans, they are associated

with increasing age (Watson et al. 2016). Oncogenic transformation of pig cells like that of humans is a rare occurrence that necessitates numerous genetic changes (Schook et al. 2015). The topic of whether duplication of human tumorigenic mutations in pigs has a similar effect on cell transformation and cancer has been a long-standing one. This appears to be the case thus far. When Adam and coworkers overexpressed tumor transgenes into pig primary fibroblast cells, the cells became tumorigenic when autologously transplanted back into the donor animals (Adam et al. 2006). To transform to a completely transmogrified phenotype, Saalfrank and coworkers investigated experimentally the stages of sarcomagenesis in vitro and discovered that porcine mesenchymal stem cells (MSCs) are similar to human MSCs in that they necessitate perturbation of the *p53*, *KRAS*, and *MYC* signaling pathways, as well as spontaneous Rb pathway inactivation and telomerase-independent immortalization steps (Saalfrank et al. 2016). This is in contrast to murine MSCs, which can be changed simply by losing their p53 function (Rubio et al. 2010). These findings imply that porcine and human oncogenesis are fundamentally comparable, yet in vitro culture, randomly integrated overexpressed transgenes, and engraftment of altered cells could all be critiqued as nonphysiological artificial approaches.

Porcine Tumor Xenograft Model

In mice, xenotransplantation of human cancer cells is well established for modeling human tumors. Patient-derived xenograft (PDX) mouse models, which are created either by subcutaneous (s.c.) or orthotopic grafting of tumor samples from human into severe combined immunodeficient (SCID) (Xu et al. 2018) mice, are currently preferred over xenografting of cell lines, which may have lost the original tumor heterogeneity over long periods of culture (Murayama and Gotoh 2019). As a result, the PDX technique is a superior predictor of human tumor activity, and murine PDX models for colon, pancreatic, and breast malignancies have been developed (Puig et al. 2013; Jun et al. 2017).

Researchers have been working to produce immunodeficient pigs which could be used in a similar xenograft system. By removing the thymus and spleen in combination with pharmacological immunosuppression, Nakayama and colleagues were able to create immunodeficient pigs (Itoh et al. 2019). Although efficient, this method is quite intrusive and can only be used to produce a small number of immunodeficient pigs. Germline alteration of genes involved in B- and T-cell development, such as the X-linked interleukin-2 receptor gamma chain gene (*IL2RG*) or the *V(D)J* recombination-activating genes, is a superior alternative (*RAGs*) (Table 2). *IL2RG* disruption has resulted in SCID pigs in several studies (Suzuki et al. 2012; Watanabe et al. 2013). These animals lacked a thymus and had a decrease or reduction of T and NK cells, but they died quickly from diseases such as pneumonia.

Pigs are a relatively novel species for cancer research. Continued advances in physiological, biochemical, immunological, and genetic data will boost their utility in biomedical research, and improved tools for manipulating germline and somatic cells will make developing novel models easier.

Table 2 Major advantages of using some animal models

Animal model	Advantages	References
Mouse	Genetic manipulation is easy	Cheon and Orsulic (2011)
	Cost-effective	
	Requires less time	
	Availability	
	Genome is similar to that of human genome (99%)	
Zebrafish	High fecundity	Hason and Bartůněk (2019)
	Short generation period (approx. 3 months)	
	External fertilization	
	Rapid embryonic development	
Pig	High homology with human genome	Schachtschneider et al. (2015)
	Anatomically, genetically, and physiologically similar to humans	
	Provide relevant information to solve complex disease	

Applications of Animal Models

Xenografts

When it is required to rely on animal model systems that closely mirror tumor growth in a human patient, xenografts are employed to address key concerns in cancer research. In comparison to cells generated in vitro, human xenografts developing in immunodeficient mice are a well-established and valuable model for investigating human tumor biology in a system that better matches the actual tumor. Xenograft studies frequently employ highly passaged cell lines that have been genetically manipulated and artificially cultivated, resulting in clonal selection that may or may not be visible in patients.

The original tumor properties such as diverse histology, clinical biomolecular signature, malignant behaviors and genotypes, tumor architecture, and tumor vasculature are thought to be preserved when xenograft tumor models are created from patient-derived tumor tissue at low passage. Primary tumor xenografts are thought to provide meaningful predictive insights into clinical outcomes when examining the efficacy of innovative cancer therapy, according to this widely held belief.

Genetically Engineered Animal Models

GM animals have indeed been generated and are now being used to supplement or, in some circumstances, replace the standard chronic rodent bioassays used to evaluate risk of cancer (Eastmond et al. 2013). To examine the involvement of

individual genes and signaling pathways in chemical carcinogenesis, a vast variety of GM animals have been utilized and/or are actively being generated.

Genetic modification methods come in use to make mice with mutation in any of its gene. Researchers can now create genetic modifications at specified periods or in specific organs using current technology. Other sorts of models, such as those that make mice sensitive to human contagious diseases such as HIV or CJD, are possible.

Before a new drug can be released into the market, it must undergo extensive testing. The effectiveness of a treatment should be examined in the most similar model to the human problem as possible, which could be a GM animal, generally a mouse that has been created to match the human state. Giving a medicine to a normal mouse may have no impact, but giving it to a mutated mouse may result in a beneficial outcome if the process works.

The development of GM pigs expressing a human gene, which might prevent acute rejection of organs transferred between pigs and people, was one of the first genetic changes of larger animals. When pig tissue is grafted into another species, antibodies in the recipient attack the donated organ, resulting in graft rejection due to the inflammatory reaction. Rejection of the transplant can be avoided by making a change to some of the proteins on cells that induce the body to mount an immunological response, known as complement control proteins.

Zebrafish: Make a Good Animal Model

A fully sequenced genome, facile genome manipulation, high fecundity, short generation period (approximately 3 months), rapid embryonic development (24 hr), and external fertilization are the most favorable traits of zebrafish. Starting with the early stages of embryogenesis, the translucent zebrafish embryo permits researchers to analyze the many stages of development. Furthermore, after 48 hours of fertilization, zebrafish embryos create complete vital organs, such as the heart, gut, and blood arteries. To study human disorders, more than 10,000 mutations in protein-coding genes have been created, as well as multiple transgenic zebrafish lines.

Another significant advantage of zebrafish is the accessibility of various strains. Furthermore, keeping a high number of zebrafish in a limited quantity of laboratory area is quite cost-effective. Although zebrafish are generally simple to handle, particular care must be taken to ensure a balanced diet and proper water quality in order to maximize fish health and growth.

Canine genomes are more comparable to human genomes than rodent genomes. Canines can develop tumors that have clinical, molecular, and histological characteristics that are similar to human cancers. Uva et al. looked at gene expression in human and canine breast cancer samples, as well as normal breast tissue, and discovered that unregulated genes present in human breast cancer were also prevalent in canine breast cancer samples. The *PTEN* gene is also lost in expression in canine breast cancer, according to Ressel et al., and similar conditions can be seen in human breast cancer (Ressel et al. 2009). Nonhuman primates are similar to humans and share many characteristics with them, including physiology, metabolism, immunity, genetics, and many others, making them a good cancer study model.

Major Limitations of Animal Models

Although there are various advantages associated with use of preclinical animal models of cancer, some major limitations for using them are mentioned below:

a) In the scientific literature, animal models have not been validated as a crucial step in biomedical research.
b) Animal research's limits and inability to provide solid forecasts for human clinical trials are becoming better recognized.
c) Because negative outcomes are frequently hidden, animal studies appear to overstate the likelihood that a treatment would be effective by roughly 30% (Sena et al. 2010).
d) Only about a third of highly cited animal research is evaluated later in human trials. Of the one-third that reaches clinical trials, only about 8% of medications successfully complete phase I (Hackam and Redelmeier 2006).
e) Experimental tumors generated in rodents are the most common preclinical instruments for new-agent screening prior to clinical testing. Despite the fact that mice are the most widely used model, they are inadequate models for the bulk of human diseases.
f) Animal models cannot be used to find a cancer cure because of crucial genetic, molecular, immunologic, and cellular differences between humans and mice.
g) Researchers discovered that transcription factor binding locations differed between humans and mice in 41% to 89% of cases in a study of over 4000 genes (Gawrylewski 2007).
h) In many cases, mouse models are used to duplicate certain processes or groups of processes within a disease but not the entire range of physiological changes that occur in humans in disease settings.
i) Poor technique and failure of the models to effectively imitate the human disease situation are likely to blame for the failure to translate from animals to humans. The problem could be embedded in the animal modeling process itself. There are no best-practice standards for animal research, unlike in human clinical trials.
j) It has been suggested that therapeutic drugs be tested not only in rodents but also in higher animal species and that randomization and outcome assessor blinding be carried out. Furthermore, trials including both genders and different age groups of animals should be designed, and all findings, both good and negative, should be published.

Conclusions

In order to equip the increasing demand of different cancer patients across the globe, personalized and precision medicine approaches using various animal models of cancer are gaining momentum for exploiting their potential. Selection of preclinical animal model is a prerequisite for its successful translation into clinic; the benefits and constrains of these models should be accessed cautiously. While doing so,

several key features should be considered including the model should imitate the spectrum of cancer stages in human beings in their initiation, promotion, and/or progression and metastasis besides the route of administration of potential drug or anticancer compound and its underlying mechanism of action, scientific validation of its translational efficacy, and the window of opportunity for the treatment. To improve the clinical outcome, further exploration of preclinical animal model of various deadly cancers is warranted to defeat this fatal disease.

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