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Review Article

Transgenic Animals: Catalysts in Drug Discovery

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ABSTRACT

Transgenic technologies have fundamentally transformed scientific research by enabling precise genetic modifications with wide-ranging applications across various fields. This review examines the application of transgenic mice across various research domains, including cancer, diabetes, cardiovascular science, neurology, gastrointestinal research, and reproductive science. We explored how these models are important in enhancing the knowledge of complex biological methods, from elucidating pathways of the disease to enhancing the quality of research outcomes in these fields. Key methodologies such as CRISPR-Cas9, TALENs, knock-out, knock-in, microinjection, and embryonic stem (ES) cell transfection are highlighted, with a focus on their impact on experimental results. Additionally, the review addresses the use of various transgenic strains in drug discovery and the challenges associated with transgenic research. By integrating findings from multiple research areas, this review underscores the transformative potential of transgenic technologies and their pivotal role in driving innovation and addressing global challenges.

Introduction

Transgenic animals, genetically engineered to express specific genes from other species, serve as crucial models for studying human diseases and testing potential therapies. The study explores the pivotal tasks of transgenic animals in accelerating drug discovery and development. It talks about new developments in this area and provides particular instances of how transgenic models have advanced therapeutic research. Through the process of transgenesis, foreign DNA sequences are introduced into the genomes of transfected cells, ensuring their incorporation and transfer to the progeny.[1] It has been possible to make genetically modified animals by fertilizing them *in-vivo* or *in-vitro* using sperm cells infused with foreign DNA.[2] Researching diseases in humans and animals, testing new drugs, and evaluating potential sources of human organs could all benefit from the use of transgenic animals as models.[3]

The genetic engineering techniques to create transgenics include:

Gene Knock-out

Gene knock-out is a technique used to inactivate a specific gene in an organism by disrupting or deleting its coding sequence, thereby preventing the production of a functional protein. Knock-out mice have become invaluable tools for geneticists aiming to understand gene function in both normal physiological homeostasis and embryonic development.[4] These models are particularly useful for investigating the underlying pathways of genetic diseases and developing potential treatments, especially when a human mutation results in protein inactivation.[5]

Gene Knock-in

Gene knock-in is a technique used to introduce a specific gene or genetic sequence into a targeted location within an organism's genome. This is achieved through methods

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such as homologous recombination or CRISPR-Cas9, which are also used for gene knock-outs. Recent advancements in knock-in technology, particularly with CRISPR/Cas9, have enabled more precise and successful gene insertions. For example, the green fluorescent protein gene has been successfully inserted into pigs using this approach.[6] The speed of these technologies now allows for the introduction of biallelic alterations in specific genes and the study of resulting phenotypes in mice within a single generation—something previously unimaginable in biology.[7] It has also been demonstrated that mice can produce therapeutically useful humanized antibodies when certain regions of the human immunoglobulin gene are knocked-in.[8]

Certainly, an animal's genome is altered by human genes linked to a certain illness. Transgenic models are incredibly useful for learning about the pathophysiology of many diseases, finding possible targets for drugs, and testing novel treatments before human clinical trials. The following are the applications and considerations in the transgenic animal research.

Disease Modeling

The evolution of medicine from skill to science is largely due to models selected for specific studies on the basis of their functional and genetic characteristics.[9,10] Genetically modified animal models have been crucial in advancing medical science by significantly enhancing our understanding of biological processes.[11] These models serve as valuable tools, mimicking disease-related mechanisms in the context of comparative medicine.[12,13] The exponential progress in medical science is, in part, due to the wide variety of transgenic species employed in research.[14] For example, to examine how an oncogene induces cancer *in-vivo*, transgenic or knock-in animals that constitutively overexpress the oncogene may be used.[15]

Pharmacokinetic Studies

The CRISPR-Cas9 technique is widely used to generate animal models for DMPK research in addition to disease models. Studies on DMPK are primarily concerned with absorption, distribution, metabolism, and excretion. Potent genetic engineering tools have significantly enhanced the ability to conduct DMPK and drug-drug interaction (DDI) studies.[16] An unparalleled scope to investigate human enzyme-catalyzed reactions has been made possible by the creation of humanized transgenic mice models.[17]

Target Validation

Transgenic animals are a valuable validation method as they allow for the observation of phenotypic outcomes, helping to clarify the dynamic impact of gene alteration. [18] Tactics used to validate newly discovered targets and support decision-making include proof-of-concept trials in humans, comprehensive drug discovery programs, or collaboration with other institutions. It is important to note that inadequate validation of therapeutic targets in the early stages has been linked to poor medication approval rates and costly clinical failures.[19-21]

Personalized Medicine

Advances in CRISPR/Cas9 genome editing have enabled the direct manipulation of zygotes, allowing for targeted gene disruption and the introduction of specific mutations.[22] Given the diversity of driver genes, CRISPR/Cas9 serves as an effective instrument for identifying these genes, which may serve as targets for future drugs.[23-25] By generating models that accurately replicate human diseases, these animals enable researchers to gain deeper insights into disease pathogenesis, identify potential therapeutic targets, and develop more precise and effective treatments.

Regulatory Considerations and Ethical Implications

The World Organization for Animal Health has emphasized that an animal's condition and its ability to adapt to its surroundings are important factors to consider. This is one of the ethical concerns raised by a significant increase in animal genetic engineering in recent years.[26] Transgenic animals used in research are subject to strict ethical standards and regulatory oversight to ensure their humane treatment and responsible use.[27]

Various transgenic models have emerged as indispensable tools in biomedical research, each offering unique advantages and insights across different areas of scientific inquiry. A few of these are outlined below:

Transgenics Used in the Cancer Research

In the cancer research, we highlighted the transgenic species used in prostate, breast, skin and pancreatic cancers.

Prostate cancer

This is currently the prevalent cancer among elderly men.[28,29] While most recent prostate tumors are benign and have a good prognosis, a small percentage can progress into aggressive, fatal tumors. This presents a significant clinical challenge in differentiating between benign and potentially life-threatening cases. The transgenic models used in prostate cancer research are (Table 1):

Tramp

Mouse transgenic adenocarcinoma TRAMP is the acronym for prostate. The TRAMP model's component is the minimum pro-basin promoter (-426/+28), which drives the viral SV40 small-t and large-T antigens. This selectively deactivates pRb and p53 in the prostatic epithelium of the prostate.[30,31] The aberrant cell proliferation in the prostate gland of TRAMP mice starts at 42 days of age and gets worse throughout the course of 168 days. By 24 weeks, almost all of the animals have invasive, poorly

differentiated adenocarcinomas, indicating that the illness is highly penetrating. [32].

- *Large T antigen driven (LADY)*

SV40 big antigen T is also used in the LADY model. According to the LADY model, the large PB promoter (LPB) is expressed by the SV40 big antigen T tag, and a deletion construct inhibits the expression of the small t antigen.[33] All things considered, the LADY model's expression of the SV40 large T antigen under the PB promoter, along with the suppression of the small t antigen, is probably going to produce a model of prostate cancer development that may differ from a model that expresses both large and small T antigens.

- *P ten knock-out*

The conditional deletion of the PTEN gene in the prostate causes cancer. According to preliminary research, homozygous Pten knock-out mice (Pten−/−) were fatal to the embryo between days 3.5 and 9.5. Nevertheless, heterozygous Pten+/− mice lived to adulthood and acquired a range of malignant growths, such as prostatic intraepithelial neoplasia (PIN) and lymphomas.[34,35] The study reveals that lowering PTEN levels below 50% increases prostate cancer progression, indicating that PTEN tumor suppression operates on a continuum, not a stepwise reduction.[36,37]

- *Nkx3.1; Pten*

This model suggests that the simultaneous deletion of the Nkx3.1 and P ten genes leads to aggressive prostate cancer. Reduced levels of both proteins result in increased prostate neoplasia since gene-targeted animal studies show that both genes work together to reduce prostate cancer. Furthermore, engineered loss of murine Pten frequently coexists with spontaneous loss of Nkx3.1 protein.[38,39] In conclusion, PTEN and Nkx3.1 are crucial tumor suppressors in the prostate. Prostate cancer may occur as a result of their loss or malfunction.

Breast cancer

Transgenic mice models are crucial for preclinical research because this malignancy is the top cause of mortality for women in America, accounting for approximately 40,000 deaths per year.[40] In order to address the high death rates linked to breast cancer, it is imperative that these preclinical models be developed. Technological developments have made it possible to produce genetically altered animals that faithfully mimic different subtypes of breast tumors.[41] The following transgenics are used to treat breast cancer (Table 2):

- *MMTV-PyMT*

The mouse mammary tumor virus-polyoma middle T antigen is known as MMTV-PyMT. In this model, the polyoma middle T antigen is influenced by the mouse mammary tumor virus promoter, which accelerates the growth of breast tumors. Many approaches, such as gene activation and suppression, made it possible to research the beginning and development of breast cancer. According to research, the middle T antigen, which contains a powerful transforming protein, is essential to the transformation process of DNA tumor viruses like the polyoma virus.[42] The progression of spontaneous mammary tumors in hemizygous MMTV-PyVT mice resembles the progression of premalignant to malignant breast cancer in humans.[43]

- *ERBB2/HER2/neu*

Another name for ERBB2, which is produced from a neurogenic strain of rats, is Human epidermal growth factor receptor 2 (HER2). The mouse mammary tumor virus-long terminal repeat (MMTV-LTR) promoter drives the active rat c-neu oncogene in this animal, creating a breast cancer model.[44]

TGFβR2 transgenic mice

This model overexpresses the ErbB2 oncogene under the MMTV promoter, leading to mammary tumor formation. TGFβR2, a threonine/serine kinase receptor, is commonly

Table 1: Uses and limitations of species used in the prostate cancer

Species	*Uses*	*Limitations*
TRAMP	Used to investigate how prostate cancer develops from its earliest stages to its metastases.	The Usage of SV40 T-antigen to induce tumors, which does not occur in human prostate cancer, making it less representative of the natural disease process in humans.
LADY	Utilized to look into the role that the HER2/neu oncogene plays in the emergence of breast cancer.	This model may not accurately mimic the normal control and diversity of HER2 expression observed in human breast cancer because it overexpresses the HER2/neu oncogene under a strong promoter.
P ten knock-out	Used to investigate the PTEN gene's function in controlling carcinogenesis.	Complete gene deletion can cause early lethality or developmental abnormalities, limiting its ability to fully model cancer progression in adult tissues.
Nkx3.1; Pten	Used to investigate the combined impact of Pten deletion and prostate-specific Nkx3.1 loss.	Nkx3.1; Pten model is that it may not fully capture the complexity and heterogeneity of human prostate cancer, as it focuses on specific genetic alterations that may not be present in all patients.

Table 2: Uses and limitations of species used in the breast cancer

Species	*Uses*	*Limitations*
MMTV-PyMT	Used to investigate how breast cancer develops, particularly how benign tumors turn into malignant ones.	It may predominantly mimic a specific subtype of breast cancer (HER2-positive), which may not represent the full diversity of breast cancer types in humans.
ERBB2/HER2/neu	Used to assess the effectiveness of targeted therapy and look into the molecular pathways underlying HER2-positive breast cancer.\	It often results in homogeneous tumor expression of HER2, which may not accurately reflect the variability and complexity of HER2 expression observed in human breast cancers.
TGFβR2	Utilized to look into the role that transforming growth factor-beta (TGF-β) signaling plays in the initiation and progression of cancer.	The genetic alterations affecting TGF-β signaling can lead to compensatory mechanisms, which may obscure the specific contributions of TGF-β in tumor biology.
MMTV-Wnt1	Used to understand the mechanisms behind tumor initiation and the influence of the tumor microenvironment.	It predominantly mimics a specific mechanism of breast cancer driven by Wnt signaling, which may not represent the heterogeneity and multifactorial nature of breast cancer in humans.
MMTV-TGFα	Utilized to investigate how transforming growth factor-alpha (TGF-α) contributes to the development of breast cancer.	This model primarily focuses on the effects of TGF-α overexpression, which may not accurately reflect the complex signaling interactions present in human breast cancer.
BRCA1/BRCA2	Used to study the molecular mechanisms underlying hereditary breast and ovarian cancers.	It may not fully capture the heterogeneity of sporadic breast and ovarian cancers, which can arise from various genetic and environmental factors beyond BRCA mutations.
MMTV-Cre; p53flox/flox	Used to help in investigation for researchers to explore how p53 loss influences tumor behaviour and response to therapy.	The deletion of p53 occurs specifically in the mammary tissue, which may not accurately represent the systemic impacts of p53 depletion observed in other tissues or malignancies.
RB1	Employed to investigate how the RB tumor suppressor gene contributes to the genesis of cancer.	It primarily focuses on retinoblastoma and may not fully represent the diverse roles of RB1 in other tumor types and the complexities of tumor microenvironments.

associated with the mechanisms underlying breast cancer. Upon binding to its ligand, Transforming growth factor beta (TGFβ), TGFβR2 heterodimerizes with TGFβR1. Substrates involved in gene transcription, cell cycle arrest, actin cytoskeleton dynamics, and cell proliferation become phosphorylated as a result of this interaction.[45,46]

- *MMTV-Wnt1*

The mouse mammary tumor virus (MMTV) promoter drives the expression of the Wnt1 oncogene, a gene involved in cell signaling and development. This model leads to mammary gland hyperplasia and tumor development. The long terminal repeat (LTR) of MMTV features multiple transcription factor binding sites that regulate viral production in various tissues and in response to hormones such as progesterone and glucocorticoids.[47,48] MMTV LTRs are crucial for initiating oncogene expression in transgenic mice, thus creating breast cancer models by regulating high-level expression of mammary epithelial cells in a hormone-dependent manner.[49] When breast tissue has Wnt1, c-neu, or TGFβ transgenes, MMTV infection raises the likelihood of cancer.[50,51]

- *MMTV-TGFα*

This model overexpresses TGFα under the MMTV promoter, leading to tumor development. TGFα stimulates the growth of epithelial and fibroblastic cells from benign tumors in healthy mammary glands.[52] TGFα promotes the proliferation of epithelial and fibroblastic cells from healthy mammary glands as well as benign malignancies. Furthermore, because TGFα and its receptor, EGF-R, are overexpressed in breast cancer cells, TGFα mediates estrogen-stimulated actions in these cells. These reactions lead to the development of breast carcinogenesis.[53]

- *BRCA1/BRCA2 knock-out*

The risk of developing cancer, particularly ovarian and breast cancer, is significantly increased by mutations in the essential genes BRCA1 and BRCA2, which are involved in DNA repair. Hereditary breast cancer has been modeled in mice by conditionally shutting down BRCA1 or BRCA2 in mammary epithelial cells. In one animal, the Brca1 exons 22–24, which encode the second BRCT domain, were precisely deleted in the mammary gland using the β-lactoglobulin (BLG) promoter. This deletion,

Table 3: Uses and limitations of species used in pancreatic cancer

Species	*Uses*	*Limitations*
Cre; Kras^G12D mice	Used to investigate the processes by which pancreatic cancer arises and spreads, with a focus on the functions of Kras mutations.	It may not accurately replicate the full spectrum of genetic mutations and heterogeneity found in human pancreatic cancer, potentially oversimplifying tumor biology.
Conditional Kras Model	Used to investigate the processes by which pancreatic cancer arises and spreads, with a focus on the functions of Kras mutations.	It may lead to variability in tumorigenesis that may not accurately reflect the progression of human cancers.

combined with a heterozygous Trp53 mutation, led to the development of breast cancers.[54]

- *MMTV-Cre; p53flox/flox*

Cre is a site-specific recombinase enzyme used in genetic models to induce targeted gene deletions. In this model, the p53 tumor suppressor gene in breast tissue is precisely deleted using the MMTV-Cre system, which results in the growth of tumors. In 1994, the Weinberg group developed the first p53 knock-out mouse model by introducing a mutation in the Tp53 gene, which blocks the translation of this gene into its functional protein.[55] In these p53-null animals, malignancies, primarily sarcomas and lymphomas, are more prevalent, with a timeframe of 10 months in p53+/– mice and 3–6 months in p53–/– mice.[56]

- *RB1*

This mice model is crucial for studying the RB1 (Retinoblastoma) role in various biological processes, particularly in cancer research. RB1 is a tumour suppressor gene, regulating the cell cycle, and its inactivation is associated with several types of cancers. The protein known as Retinoblastoma Protein 1(RB1) controls the progression of the cell cycle.[57]. Luminal B and basal-like breast cancers have been linked to RB1 loss or inactivation.[58,59]

Pancreatic cancer

The most prevalent and possibly lethal malignancy in adults is pancreatic cancer.[60] Transgenic models offer a good understanding of the disease pathways, particularly the abnormal tumor microenvironment that fosters cancer growth, as well as genetic alterations and acquired traits of cancer cells. The following transgenic models are employed in the study of pancreatic cancer (Table 3).

- *Cre; Kras^G12D mice*

A specific mutation known as the "Kras^G12D mutation" occurs in the Kras gene, causing aspartic acid (D) to replace glycine (G) at position 12 in the Kras protein. This mutation, which is a common oncogenic driver in a range of malignancies, including colorectal, lung, and pancreatic cancers, is a crucial model for studying oncogenic Kras-driven carcinogenesis. Kras^G12D is frequently mutated in pancreatic carcinogenesis. The oncogenic Kras^G12D allele, when combined with the Cre-loxP system, produces cancer in a tissue-specific manner in this model. This model causes tumors in mice that mimic pancreatic ductal adenocarcinoma (PDAC) and intraductal papillary mucinous neoplasm (IPMN). By evaluating tumors from Ptf1a-Cre, Kras^G12D, and Arid1a^f/f mice, Wang *et al*. showed that these cancers activated the epithelial-mesenchymal transition (EMT) and stem cell identity pathways.[61,62]

- *Conditional kras model*

Conditional Kras models are genetically engineered animals or cell lines designed to allow controlled expression or inactivation of the Kras oncogene in specific tissues or developmental stages. The use of the Cre-loxP system or similar recombination technologies achieves this control. These models are useful for researching how oncogenic Kras mutations affect the onset and spread of cancer. Pancreatic ductal cancer arises when LSL-Kras^G12D mice are crossed with mice that express Cre recombinase, which is controlled by the Pdx1 or Ptf1a/P48 promoters.[63] The KRAS mutation, which is seen in almost all human pancreatic ductal adenocarcinomas (PDACs), is one of the initial genetic events in the development of human pancreatic intraepithelial neoplasms (PanINs).[64] Further research using murine models has shown that activation of a KRAS mutation is the initial step toward PDAC development.[65,66]

Skin cancer

Transgenic models are invaluable for studying gene interactions and their impact on skin cancer onset and progression. For over 60 years, multistage skin carcinomas in mice have served as a reliable *in-vivo* model for investigating epithelial tumor development.[67,68] Below are some of the models employed in skin cancer research (Table 4):

- *K14-HPV16 mice*

K14 is a protein expressed in basal keratinocytes, the primary cell type in the epidermis, while HPV16 is a high-risk type of human papillomavirus associated with skin and cervical cancers. In this model, the HPV16 genome has been introduced into the genome of mice (HPV+), resulting in basal cells of keratinized epithelia expressing the HPV16 early region.[69] This expression is induced by the promoter of the cytokeratin-14 (K14) gene.[70] The

Table 4: Uses and limitations of species used in the skin cancer

Species	*Uses*	*Limitations*
K14-HPV16	Used to investigate how human papillomavirus, or HPV, contributes to the development of skin cancer.	It predominantly focuses on HPV16-related tumors, which may not capture the full spectrum of HPV-associated cancers and their diverse genetic backgrounds in humans.
MMTV-FLAG-PAD2	Utilized to investigate how peptidyl arginine deiminase 2 (PAD2) leads to the development of breast cancer.	Overexpression of PAD2 may not accurately represent its physiological regulation in normal breast tissue, potentially leading to atypical tumor characteristics not seen in human cancers.
Tyr-NRASQ61K	used to look at how NRAS mutations affect the onset and spread of melanoma.	It primarily focuses on the effects of a single oncogenic mutation, which may not fully capture the genetic and environmental complexity of human melanoma.

epithelial cells in these animals are specifically targeted for HPV16 early region expression due to the influence of the cytokeratin-14 gene promoter.[71]

- *MMTV-FLAG-PAD2 mice*

While peptidyl arginine deiminase 2 (PAD2) is an enzyme involved in protein modification, FLAG is a short peptide tag used for protein purification and detection. A useful tool for researching how protein arginine deiminase 2 contributes to skin cancer is PAD2. By subcloning the human PAD2 cDNA into the EcoRI sites of the MMTV-SV40-Bssk plasmid, scientists create the MMTV-FLAG-PAD2 construct.[72,73] This construct enables targeted expression and study of the PAD2 protein, specifically in mammary cells.

- *Tyr-NRASQ61K mice*

The Tyr promoter drives the expression of the mutant NRAS gene specifically in melanocytes, while Q61K denotes a mutation in the NRAS gene where the glutamine (Q) at position 61 is replaced by a lysine (K). Tyr-NRASQ61K is used to investigate the role of NRAS^Q61K mutation in melanoma genesis. The NRAS^Q61K mutation, commonly found in human melanomas, drives oncogenesis through the activation of the NRAS protein, leading to uncontrolled cell proliferation. Mutations in BRAF (51–63%) and NRAS (21–28%) are responsible for up to 75 to 80% of melanomas, often working in tandem.[74] In 1995, the transgenic mouse model known as TP-Ras was developed, which overexpresses the mutant human HRAS^G12V under the Tyr promoter. In this model, melanocytes did not develop into melanoma despite showing hyperpigmentation and hyperproliferation.[75] Chin *et al*. found in 1997 that recurring skin-pigmented tumors with 61% penetrance are produced when HRAS^G12V is expressed selectively in melanocytes under the Tyr promoter in a homozygous Ink4a/Arf-null background.[76]

Transgenics Used in the Diabetic Research

For the purpose of studying the impact of diabetes on metabolism, fertility, death rate and illness rate across multiple organs, global knock-out or knock-in mice are essential.[77,78] Transgenic models have also been utilized to investigate the potential for liver-to-pancreas redirection in autoimmune pancreatitis, offering a more realistic depiction of the disease's progression.[79] The following transgenic models may be particularly useful for diabetes research (Table 5).

Lepr^db (db/db) Mice

Mutation in the leptin receptor gene causes obesity and severe diabetes in these animals. They are particularly valuable for studying type 2 diabetes (T2D) and its complications. Lepr mutations result in obesity in Lepr^db/db mice,[80,81] which is akin to the obesity observed in leptin-deficient ob/ob animals.[82] Additionally, Lepr^db/db mice develop insulin resistance, fatty liver, hyperphagia, hyperglycemia, and infertility.[83,84]

Non-obese diabetic (NOD) mice

This model is used for studying type 1 diabetes (T1D). In the 1980s, researchers in Japan sought to breed a strain of mice susceptible to cataracts, but instead, they spontaneously developed type 1 diabetes at a young age. This unintentional development led to the creation of the NOD mice model.[85] It is now understood that, akin to human T1D, the autoimmune destruction of the endocrine pancreas in NOD mice leads to diabetes. This destruction results in insulin insufficiency and elevated blood sugar levels, or hyperglycemia.[86]

KK-A^y mice

Because the A^y allele is linked to increased body weight and metabolic abnormalities, KK-A^y mice are frequently utilized as models for obesity and metabolic disorders. Genetic and environmental variables that lead to obesity and associated disorders can be investigated using these mice. Agouti gene mutation, responsible for hyperphagia, develops diabetes. A consistent diabetic state may be produced in Kuo Kondo (KK/Ay) mice by the use of a polygenic mutant model of T2D. This mouse model also tackles the issue of there not being a good T2D model available for bone investigations. Agouti gene mutation, which determines a mouse's coat color, causes KK/Ay to have diabetes. Agouti gene mutation coats ectopic gene expression in various tissues, which leads to diabetic mice with yellow fur.[87]

C57BLKS/J (BKS)

A particular inbred mouse model called the C57BLKS/J (BKS) strain is frequently used to research obesity and type 2 diabetes (T2D). Early-onset diabetes is caused by a recessive mutation in this strain that develops on its own. Initially, the mutation causes mild hyperinsulinemia, which progresses to insulinopenia due to beta-cell degeneration in the pancreatic islets.[88] In 1996, it was discovered that this strain also exhibits a mutation in the leptin receptor, a key adipokine hormone. This mutation is found in ob/ob (now Lepob) mice, furthering our understanding of diabetes and obesity.[89-91]

NONcNZO10/LtJ

The NONcNZO strain, which is a combination of the NON, NZO, and C57BL/6 strains, includes substrain 10 and is identified by the Jackson Laboratory stock number LtJ. This strain is utilized to study the complex genetic relationships involved in diabetes. Polygenic models of obesity and diabetes, like NONcNZO, more accurately reflect the genetic diversity seen in human type 2 diabetes (T2D) patients. NZO/HlJ mice, a related strain, develop severe obesity and insulin resistance while maintaining a functional leptin-leptin receptor axis, indicating peripheral resistance rather than central involvement. These mice exhibit abnormalities similar to those observed in BKS-db/db mice.[92-94]

TALLYHO mice

TALLYHO is a transgenic mouse model in which the human telomerase reverse transcriptase (hTERT) gene expression is controlled by the mouse mammary tumor virus (MMTV) promoter. Type 2 diabetes in this polygenic model results in insulin resistance and hyperglycemia. Male diabetic mice have been used to inbreed and select offspring, creating a polygenic diabetes model that resembles NcZ10. Male TALLYHO diabetic mice have pancreatic islet histopathology that shows beta-cell degranulation and loss.[95,96]

OB/OB mice

Mice with a mutation in the leptin gene develop fat and diabetes because alterations in leptin signaling are closely linked to metabolic issues and the onset of type 2 diabetes mellitus (T2DM).[97] Obesity and related metabolic disorders are frequently studied in genetically defective ob/ob mice.[98,99] While the diabetes (db) gene, which codes for the leptin receptor (ObR), is altered in db/db mice, the obesity (ob) gene, which codes for leptin, is mutated in these mice.[100] Ob/ob animals mainly show obesity and mild insulin resistance, whereas db/db mice acquire both diabetes and obesity.[101]

Transgenics Used in the Cardiovascular Research

The intricate network of hormonal and neurological pathways that influence organ function is critical for

Table 5: Uses and limitations of species used in diabetic research

Species	*Uses*	*Limitations*
Lepr^db (db/db)	Used to study type 2 diabetes, making it valuable for researching metabolic disorders and potential therapeutic interventions.	It mostly depicts type 2 diabetes and monogenic obesity, which might not accurately portray the complex etiology of these disorders in people.
NONOBESE DIABETIC (NOD)	Used to investigate the pathophysiology of type 1 diabetes, specifically how autoimmune contributes to pancreatic cell destruction.	It might not accurately mimic the environmental and genetic elements that lead to the onset of type 1 diabetes in people.
KK-A^y	Employed to look into how insulin resistance and hyperphagia affect type 2 diabetes and the metabolic syndrome linked to obesity.	Severe obesity is driven by a specific genetic mutation, which may not fully represent the diverse causes of type 2 diabetes in humans.
C57BLKS/J (BKS)	Used to investigate severe diabetes and obesity, especially when combined with mutations in the leptin receptor or leptin itself.	The susceptibility to severe obesity and diabetes is primarily due to specific genetic backgrounds, which may not fully capture the complexity of these conditions in the broader human population.
NONcNZO10/LtJ	Used to study polygenic obesity, metabolic syndrome, and Diabetes type 2.	The polygenic nature of this model can make it challenging to pinpoint specific gene contributions, complicating the study of individual factors involved in obesity and Diabetes type 2.
TALLYHO	Used to investigate the pathophysiology of Diabetes type 2 and obesity, particularly the interactions between genetic predisposition.	This model exhibits a specific genetic background, which may not fully represent the multifactorial and heterogeneous nature of diabetes type 2 in human populations.
ob/ob	Used to study obesity and metabolic disorders, particularly body weight regulation.	It primarily reflects a single genetic defect in leptin signaling, which may not capture the complexity of obesity's multifactorial causes in humans.

cardiovascular control and the etiology of cardiovascular disorders.[102] Transgenic technology plays a vital role in characterizing genes associated with cardiovascular health. However, its gene-targeting techniques are currently limited to mouse species, which presents a significant challenge.[103] Below are some transgenic models used in cardiovascular research that are worth considering (Table 6):

ApoE knock-out mice (ApoE−/−) & LDL receptor knock-out mice (LDLR−/−)

These ApoE-deficient animals are utilized to simulate human atherosclerosis and hypercholesterolemia, much as ApoE knock-out mice. A new era in the preclinical study of atherogenesis began in the early 1990s with the description of the apoprotein E deficient mice (Apoe−/−) by N. Maeda and colleagues and by J. Breslow and colleagues in the same year. The following year, Herz, Brown, and Goldstein described the LDL receptor-deficient mouse (Ldlr−/−).[104-106] Apoe−/− mice are utilized more often than Ldlr−/\ animals, but both models—which have the atherosclerosis-prone C57BL/6 genetic background—are widely employed to study atherosclerosis utilizing a variety of physiological and genetic interventions. The LDL receptor is downregulated when hyperlipidemia is present, and transplanting bone marrow expressing Ldlr has little to no effect on atherosclerosis overall in animals that are Ldlr-deficient.[107] Both the Apoe−/− and the Ldlr−/− mice have been used to assess the role of a large number of genes in atherogenesis.[108, 109] Mice that are fed a Western-style diet experience hyperlipidemia-related atherosclerosis.[110] Obstructive lipid-rich coronary lesions associated with myocardial infarction are also caused by the overexpression of urokinase specific to macrophages on an Apoe−/− background.[111]

PKC-delta Knock-out Mice (PKC-δ−/−)

These mice lack protein kinase C-delta and are used to study ischemic heart disease. Genetic variants exist in PKC-δ, and the PKC-δ gene, Prkcd, can be spliced to create conditional knock-out mice. Controlling exon expression results in the production of these mice. These mice were created by removing common exons of various PKC-δ genes I, II, IV, V, VI, and VII. PKC-δ−/− mice used in these investigations are primarily PKC-δ I, II subtypes that have been knocked out. Niino *et al*.'s study found that knocking down various PKC-δ subtypes in mice can inhibit fetal development, cause lung inflammation, and lead to heart elastic fiber hyperplasia in adult mice.[112]

Transgenic mice overexpressing human renin and angiotensinogen

These mice are used to study hypertension and its effects on cardiovascular health. Developed by the Indiana University School of Medicine, the DBA/2N transgenic mice are specifically designed to investigate hypertension and its impact on cardiovascular health [113]. Initially, transgenic mice expressing human renin were created by inserting linear DNA segments containing either the full human renin gene (15.3 kbp) or the complete human angiotensinogen gene (14 kbp) into C57BL/6J ova. Subsequently, these two heterozygous mice were crossed to generate the desired transgenic model.

MHC-α/β transgenic mice

These mice express mutant forms of MHC and are used to study cardiac hypertrophy and heart failure. GATA4 knock-out mice showed overt hypertrophy and heart failure, linked to increased βMHC (beta-myosin heavy chain) and ANF (atrial natriuretic factor) expression. These observations challenge the idea that GATA4 induction directly influences βMHC expression during pressure-overload hypertrophy.[114] The creation of these mice involves precise genetic engineering techniques to introduce foreign MHC genes into the mouse genome.

Transgenics Used in the Neurological Research

The use of transgenic animals expressing fluorescent reporters for imaging studies has significantly increased with the advancement of recombinant DNA technology. These models enable vivid imaging through fluorescence microscopy techniques, such as confocal microscopy, allowing for the visualization of physiological and pathological events within the nervous system. This approach has been instrumental in studying conditions such as Huntington's disease,[115] Alzheimer's disease (AD),[116, 117] Parkinson's disease,[118] and amyotrophic lateral sclerosis.(ALS)[117] Additionally, considerable efforts have been directed toward developing novel transgenic models to enhance our understanding of less well-characterized diseases, such as schizophrenia.[119] Here are some examples of well-known transgenic models used in neurological research:

APP/PS1 mice

Human amyloid precursor protein (APP) and presenilin 1 (PS1), genes linked to familial AD, a genetic variant of the neurodegenerative disease, are overexpressed in APP/PS1 mice. These mice produce a chimeric APP695 protein with Swedish mutations (K595N, M596L), which is controlled by the MMP promoter. Additionally, the PS1 variant in these mice includes the ΔE9 deletion mutation. This model is known as the APP(Swe)/PS1ΔE9 model.[120]

B6-hSNCA mice

The B6-hSNCA mouse model carries a humanized version of the SNCA gene, which encodes α-synuclein, making it valuable for Parkinson's disease research. Mutations in SNCA gene lead to the overproduction of α-synuclein, which contributes to the formation of Lewy bodies. This model provides a humanized system to study the SNCA gene, a promising target for developing new treatments.

Table 6: Uses and limitations of species used in the cardiovascular research

Species	*Uses*	*Limitations*
(ApoE−/−) & (LDLR−/−)	Used to study atherosclerosis and cardiovascular disease, particularly the mechanisms of lipid metabolism and plaque formation in a hyperlipidemic environment.	They do not fully replicate the complexities of human lipid metabolism and cardiovascular disease, as these models are based on specific genetic mutations that may not encompass all contributing factors.
(PKC-δ−/−)	Used to investigate cancer progression and inflammation in cardiovascular diseases.	The complete knock-out of PKC delta can lead to compensatory mechanisms in other signaling pathways, potentially obscuring its specific role in disease processes.
Overexpressed human renin and angiotensinogen	Used to study the pathophysiology of hypertension and cardiovascular diseases.	They may not accurately replicate the complex regulatory mechanisms of the renin-angiotensin system found in humans, potentially leading to oversimplified interpretations of hypertension and cardiovascular disease.
MHC-α/β	Used to investigate the immunological response, specifically the involvement that major histocompatibility complex molecules play in autoimmunity and T cell activation.	It may not fully replicate the complexity of human immune responses due to differences in MHC allele diversity and interactions with other immune components.

The homozygous B6-hSNCA mice are both viable and reproducible.[121]

FVB/N mice

This strain is commonly used for creating transgenic models in research related to gene functions associated with some neurological diseases due to its large oocytes and pronuclei, which are ideal for genetic manipulation. Tg2576 mice are typically studied when they possess both C57BL/6 and SJL genetic backgrounds. However, when the Tg2576 gene is introduced into the FVB/N strain, the mice tend to die early, and it becomes challenging to remove the special gene from this type of mouse.[122]

Tau P301L mice

express the human tau protein with the P301L mutation linked to AD. Tauopathy patients display MAPT (microtubule-associated protein tau) clumps in their brains, causing tauopathies like AD, and FTLD.[123] The harmful tau protein, when put into the brains of mice with the human tau gene or even normal mice, can clump together.[124-126] Studies have shown that both the normal tau gene and the P301L mutation lead to increased tau protein production in mice. However, the formation of tau tangles, as seen in humans with the normal tau gene, occurs more slowly.[127] The P301L mutation causes tau protein to misfold and aggregate into neurofibrillary tangles (NFTs), which are a hallmark feature of frontotemporal dementia (FTD).

5xFAD mice

The 5xFAD mouse strain was developed to model AD by incorporating five mutations associated with the disorder, which significantly increases the production of Aβ42. This strain serves as a rapid model for studying Alzheimer's amyloid pathology. By the age of 2 months, these mice exhibit substantial accumulation of Aβ42, formation of cerebral amyloid plaques, gliosis, and memory deficits, as evidenced by the Y-maze test. Notably, intraneuronal Aβ42 accumulation occurs prior to plaque formation, suggesting that amyloid plaques may originate from aggregated neurons.[128, 129]

The hSOD1 G93A mice

The hSOD1^G93A mouse model expresses the human superoxide dismutase 1 (SOD1) gene with a G93A mutation and is widely utilized to study ALS. These transgenic mice carry multiple copies of the faulty SOD1 gene, leading to a disease phenotype similar to ALS, characterized by damage to the motor neurons that control muscle function. Mice with elevated SOD1 levels exhibit a range of neurodegenerative symptoms, including axonal degeneration in several long fiber tracts, particularly the spinocerebellar tracts, and axonal swelling.[130] hSOD1WT mice show neurodegenerative alterations, including vacuolization of mitochondria in the spinal cord, brain stem, and subiculum axons, moderate spinal motoneuron loss at 2 years, and modest motor impairments.[131, 132]

Transgenics Used in the Gastrointestinal Research

Transgenic models have significantly advanced our knowledge of the pathophysiology of diseases and their cellular pathways, including gastric precancerous lesions (GPLs). As the fifth most common disease worldwide, gastric cancer significantly raises the death rate from cancer. Over 1 million people receive a GC diagnosis each year, and over 720,000 of them pass away from the illness.[133,134] By creating mice with specific genetic alterations, such as knock-out or transgenic mice, scientists have gained valuable insights into the roles of genes within living organisms and the genetic factors contributing to health and illness.[135] The genetically modified species commonly used in gastrointestinal research include (Table 8):

Table 7: Uses and Limitations of species used in the neurological research

Species	*Uses*	*Limitations*
APP/PS1	Focusses the effects of amyloid-beta accumulation on neuronal function and cognitive impairment.	It primarily focuses on amyloid-beta pathology and may not fully represent the complex nature of Alzheimer's disease, including tau pathology and neuroinflammatory processes.
B6-hSNCA	Used to investigate alpha-synuclein overexpression on neurodegeneration and motor deficits.	The complete range of Alzheimer's disease pathology, including the existence of non-motor symptoms and the complex interaction of environmental factors, might not be fully replicated by it.
FVB/N	Allows the researchers to explore its effects on neuronal toxicity and motor function.	It primarily expresses human alpha-synuclein, which may not fully recapitulate the post-translational modifications and aggregation patterns observed in human Alzheimer's disease.
Tau P301L	Used to study the pathophysiology of tau-related neurodegenerative diseases, allowing researchers to explore tau aggregation, neuroinflammation, and cognitive decline.	It may not fully replicate the heterogeneity and complexity of human tauopathies, as it primarily focuses on a specific mutation and its downstream effects.
5xFAD	Used to study the pathophysiology of Alzheimer's disease, particularly the rapid buildup of amyloid-beta plaques.	It develops amyloid plaques rapidly, which may not accurately reflect the slower, more progressive plaque formation observed in human Alzheimer's disease.
hSOD1 G93A	Used to assess possible treatment strategies and look into the mechanisms of ALS.	It predominantly represents a specific genetic form of ALS, This might not fully encompass the range of pathophysiological processes at play in isolated instances of the illness.

The APC^min /+ Mouse model

Many intestinal adenomas, mostly in the small intestine, occur as a result of a mutation in the APC gene present in the APC^min /+ Mouse model. Mice with a heterozygous APC mutation are able to live but develop numerous intestinal polyps.[136] Referred to as "Min" mice, or multiple intestinal neoplasia mice, they serve as valuable models for studying intestinal tumorigenesis due to their polyps resembling those found in patients with familial adenomatous polyposis (FAP).[137] Additionally, COX-2, a gene responsible for promoting intestinal and colonic polyps, was found to significantly reduce the number of intestinal polyps in APC^Min mice when Δ716 animals were given COX-2 (Ptgs2 null mutation) knock-outs.[138]

CAC

Cre recombinase was particularly expressed in the large intestine of the CAC mouse model. This model makes use of a promoter from the mouse carbonic anhydrase I gene. This transgenic animal was created by crossing CAC mice with APC580S mice, which inactivated the APC gene in either one allele (CAC; APC580S/+) or both alleles (CAC; APC580S/580S). Nonetheless, the small intestine of Apcmin mice and tissues beyond the large intestine frequently exhibit increased malignancy expression in the transgenic models for colon cancer that are currently available.[139] Additionally, Apcmin mice and other genetically modified mice have been found to develop mammary tumors.[140] Genetically modified mice allow for precise control over cancer initiation molecular mutations. The CAC model was generated using Pme I restriction endonuclease digestion to separate CAC from pUC13kb-mCA-cre, following conventional procedures at the Purdue University Transgenic Mouse Core Facility.[141]

cMyc and shp53 tansgenic mice

The cMyc and shp53 transgenic mice model overexpresses cMyc and exhibits reduced p53 expression in the liver, serving as a valuable model for studying hepatocellular carcinoma (HCC) and gastrointestinal cancer. Developed by Sandgren *et al*., these mice utilize an albumin enhancer/promoter to drive c-Myc expression, specifically in the liver. This targeted expression leads to the development of hepatoblastoma in older mice after 15 months, while younger mice display varying degrees of liver damage.[142] The study demonstrated that c-Myc overexpression results in β-catenin gene mutations, which disrupt β-catenin signaling pathways and ultimately contribute to HCC development.[143]

Transgenics Used in the Reproductive Research

For the past 20 years, transgenic mice have advanced our knowledge of how transcription factors regulate the reproductive system. Their genetic and physiological similarities to humans, coupled with their ease of care, rapid breeding, and high reproductive rates, make mice the most popularly used ones in biomedical research. The availability of the mouse genome shortly after the human genome further underscores their importance in health science research.[144, 145] Some notable transgenic models that have contributed to reproductive research include (Table 9):

Table 8: Uses and limitations of species used in the gastrointestinal research

Species	*Uses*	*Limitations*
APC^min /+	used to investigate how APC mutations influence intestinal tumor development and progression.	This model develops tumors in the small intestine, whereas human colorectal cancer typically arises in the colon, limiting its direct relevance to human disease.
CAC	The connection between chronic inflammation and colon cancer is investigated using the colitis-associated cancer (CAC) model, which sheds light on the mechanisms behind inflammation-driven carcinogenesis.	The induced inflammation and tumor development may not fully mimic the gradual and multifactorial progression of colitis-associated cancer in humans.
cMyc and shp53	used to investigate how p53 suppression and MYC overexpression work together to promote carcinogenesis, especially in lymphoma cancer.	It may not fully capture the genetic diversity and heterogeneity found in human cancers, limiting its ability to represent all tumor subtypes.

Table 9: Uses and limitations of species used in the reproductive research

Species	*Uses*	*Limitations*
C57BL/6J	s	Its susceptibility to certain metabolic conditions may not be representative of responses in other mouse strains or in humans.
BALB/c	Used in studying tumor development and infectious diseases due to its Th2-biased immune system.	The limitation of the BALB/c model is its genetic uniformity, which may not accurately reflect the heterogeneity of immune responses and disease progression seen in diverse human populations.
CD-1	The CD-1 mouse model is widely used in pharmacological and toxicological studies due to its high reproductive capacity.	A limitation of the CD-1 model is its genetic variability, which can lead to inconsistent results in experiments compared to more genetically uniform strains.
FVB/N	The FVB/N model is used for transgenic studies due to its high reproductive performance and favorable conditions for germline transmission.	A limitation of the FVB/N model is its susceptibility to certain diseases, which can complicate experimental outcomes and affect the generalizability of findings.

C57BL/6J

strain is The C57BL/6J strain is widely utilized due to its well-defined genome and robust reproductive performance. C57BL/6J mice are a versatile and popular strain in biomedical research. Their combination of genetic homogeneity, robustness, and disease susceptibility makes them well-suited for a variety of applications.

BALB/c

This strain is frequently utilized for generating transgenic lines to study reproductive toxicology and fertility effects due to its strong reproductive capabilities.

CD-1 strain is widely utilized in reproductive studies due to its high fertility rates and large litter sizes, making it ideal for embryo transfer and cryopreservation studies.

FVB/N is favored for pronuclear microinjection due to its large oocyte and pronuclei size, making it a popular choice for creating transgenic mice expressing reporter genes or reproductive research modifications. FVB/N mice are used as inbred strains in biomedical research. They are known for their high fertility, rapid growth rate, and relative ease of handling.

Ethical Implications in the Welfare of Transgenic Animals

Several ethical issues are brought up by the use of transgenic animals in drug discovery:

Animal welfare

The use of transgenic animals often involves procedures that can cause pain, suffering, or distress. Ethical considerations focus on minimizing harm and ensuring humane treatment, including appropriate housing, care, and the use of anesthesia or pain relief during procedures.

Necessity and justification

There must be a strong scientific justification for using transgenic animals. Researchers need to demonstrate that the knowledge gained or the potential benefits of drug discovery outweigh the ethical costs.

Genetic alteration consequences

The genetic modifications in transgenic animals may have unforeseen consequences, affecting not just the individual animal's well-being but potentially leading to broader ecological impacts if such animals were to be accidentally released into the wild. The long-term welfare of genetically modified animals must be considered.

Regulatory oversight

The creation and use of transgenic animals are subject to strict regulatory frameworks designed to ensure ethical standards are met. These include institutional review boards and ethics committees that assess research protocols to ensure they align with ethical guidelines.

Public perception and trust

The use of transgenic animals can raise public concerns about the ethics of genetic modification, animal rights, and the natural order. Researchers must engage with the public transparently, explaining the purpose, benefits, and ethical safeguards in place to build trust.

Moral status of animals

Some ethical frameworks question whether it is morally acceptable to create transgenic animals for human benefit, considering the animals' potential suffering and the alteration of their natural state.

Impact on biodiversity

The creation of transgenic animals could potentially impact natural biodiversity if these animals were to interact with wild populations. The ethical implications of such an impact need to be carefully evaluated, with appropriate containment and monitoring measures in place.

Challenges

Genetic background variability

The genetic background of an animal can greatly impact the phenotype of a transgenic model, making it challenging to differentiate the effects of the transgene from background genetic variation. Maintaining a stable and consistent genetic background requires careful breeding and management, as inbreeding or genetic drift can introduce variability over time.

Ethical and social considerations

Public concerns about genetic modification and animal welfare can lead to resistance against use of transgenic animals, affecting funding, regulatory approval, and societal support. Researchers must balance the scientific benefits of using transgenic animals with the ethical obligation to minimize harm and justify the necessity of such models.

Environmental and biosafety risks

Ensuring that transgenic animals do not escape or interbreed with wild populations is critical to preventing the spread of altered genes into the environment. The long-term effects of genetic modifications on animal health, behavior, and ecosystems are not fully understood, posing risks that require careful monitoring and management.

Reproducibility and validation

Variability in the creation and maintenance of transgenic models can lead to reproducibility challenges, making it difficult to replicate and validate experimental findings. Establishing standardized protocols for generating and using transgenic animals is essential to ensure consistency and reliability in research outcomes.

Alternative models and technologies

Advances in alternative research methods, including organoids and CRISPR-based gene editing, are offering new tools that could lessen the dependence on transgenic animals. Ethical and legal frameworks are placing greater emphasis on challenging the continued use of transgenic animals in research by focusing on the reduction, refinement, and replacement (3Rs) of animal use.

Limitations

Complexity of genetic modifications

Genetic modification can lead to off-target effects or unintended changes in the genome, potentially causing unexpected phenotypes or health problems in transgenic animals. The expression of the introduced gene may vary among individuals, leading to inconsistent results in experiments. The introduced gene might be silenced or expressed at lower levels than intended, making it difficult to study the gene's function or effect accurately.

Species-specific differences

Findings from transgenic animal models may not always translate directly to humans due to species-specific differences in genetics, physiology, and disease mechanisms. The physiological and behavioral differences between animals and humans can complicate the interpretation of experimental results, limiting the applicability of findings to human health.

High costs and time-intensive processes

Creating and maintaining transgenic animals is expensive and requires significant time, specialized facilities, and skilled personnel. The process of developing a transgenic animal model, including breeding and validation, can take several months to years, slowing down research timelines.

Ethical and regulatory constraints

The use of transgenic animals raises ethical concerns regarding animal welfare, the moral implications of genetic manipulation, and the potential suffering resulting from genetic modifications. Strict regulations oversee the invention and use of transgenic animals, potentially delaying research progress and adding complexity to the process of obtaining experimental approval.

Technical challenges

The process of integrating foreign genes into the animal genome is not always efficient, leading to low success rates and the need for repeated attempts. Precisely controlling the timing, location, and gene expression level in transgenic animals remains a challenge, which can affect the outcomes and reproducibility of experiments.

Conclusion

In conclusion, the advent of transgenic technologies has given rise to a remarkable understanding of intricate

biological systems and the causes of disease. The application of advanced methodologies like CRISPR-Cas9, TALENs, and various gene transfer techniques has enabled researchers to create precise and informative models, significantly advancing our understanding across multiple research fields. The review demonstrates that transgenic mice are indispensable tools in elucidating disease processes, improving experimental accuracy, and facilitating drug discovery. Despite the challenges associated with transgenic research, including technical and ethical considerations, the benefits and potential of these technologies are immense. By bridging gaps in knowledge and driving innovation, transgenic models are crucial for addressing both fundamental scientific questions and pressing global health issues.

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References

1. Shakweer WME, Krivoruchko AY, Dessouki SM, Khattab AA. A review of transgenic animal techniques and their applications. J Genet Eng Biotechnol. 2023;21(1):55. Available from: http://dx.doi.org/10.1186/s43141-023-00502-z
2. Shakweer WME, Hafez YM, El-Sayed AA, Awadalla IM, Mohamed MI. Construction of ovine GH-pmKate2N expression vector and its uptake by ovine spermatozoa using different methods. J Genet Eng Biotechnol. 2017;15(1):13–21. Available from: http://dx.doi.org/10.1016/j.jgeb.2017.04.001
3. Houdebine L-M. Use of transgenic animals to improve human health and animal production. Reprod Domest Anim. 2005;40(4):269–81. Available from: http://dx.doi.org/10.1111/j.1439-0531.2005.00596.x
4. Cacheiro P, Spielmann N, Mashhadi HH, Fuchs H, Gailus-Durner V, Smedley D, et al. Knockout mice are an important tool for human monogenic heart disease studies. Dis Model Mech. 2023;16(5). Available from: http://dx.doi.org/10.1242/dmm.049770
5. Hall B, Limaye A, Kulkarni AB. Overview: generation of gene knockout mice. Curr Protoc Cell Biol. 2009;19:19–31. Available from: https://doi.org/10.1002/0471143030.cb1912s44
6. Li G, Zhang X, Wang H, Mo J, Zhong C, Shi J, et al. CRISPR/Cas9-mediated integration of large transgene into pig CEP112 locus. G3 (Bethesda). 2020;10(2):467–73. Available from: http://dx.doi.org/10.1534/g3.119.400810
7. Wang Y, Li J, Xiang J, Wen B, Mu H, Zhang W, et al. Highly efficient generation of biallelic reporter gene knock-in mice via CRISPR-mediated genome editing of ESCs. Protein Cell. 2016;7(2):152–6. Available from: http://dx.doi.org/10.1007/s13238-015-0228-3
8. Verkoczy L, Alt FW, Tian M. Human Ig knockin mice to study the development and regulation of HIV-1 broadly neutralizing antibodies. Immunol Rev. 2017;275(1):89–107. Available from: http://dx.doi.org/10.1111/imr.12505
9. Hobson-West P, Davies A. Societal sentience: Constructions of the public in animal research policy and practice: Constructions of the public in animal research policy and practice. Sci Technol Human Values. 2018;43(4):671–93. Available from: http://dx.doi.org/10.1177/0162243917736138
10. Robinson NB, Krieger K, Khan FM, Huffman W, Chang M, Naik A, et al. The current state of animal models in research: A review. Int J Surg. 2019;72:9–13. Available from: http://dx.doi.org/10.1016/j.ijsu.2019.10.015
11. Lee KH, Lee DW, Kang BC. The "R" principles in laboratory animal experiments. Lab Anim Res. 2020;36(1):45. Available from: http://dx.doi.org/10.1186/s42826-020-00078-6
12. Baptista J, Faustino-Rocha AI, Oliveira PA. Animal Models in Pharmacology: A Brief History Awarding the Nobel Prizes for Physiology or Medicine. Pharmacology. 2021;106(7–8):356–68. Available from : https://doi.org/10.1159/000516240
13. Swearengen JR. Choosing the right animal model for infectious disease research. Animal Model Exp Med. 2018;1(2):100–8. Available from: http://dx.doi.org/10.1002/ame2.12020
14. Fernandes MR, Pedroso AR. Animal experimentation: A look into ethics, welfare and alternative methods. Rev Assoc Med Bras . 2017;63(11):923–8. Available from: http://dx.doi.org/10.1590/1806-9282.63.11.923
15. Porret A, Mérillat A-M, Guichard S, Beermann F, Hummler E. Tissue-specific transgenic and knockout mice. Methods Mol Biol. 2006;337:185–205. Available from: http://dx.doi.org/10.1385/1-59745-095-2:185
16. Li Y, Meng Q, Yang M, Liu D, Hou X, Tang L, et al. Current trends in drug metabolism and pharmacokinetics. Acta Pharm Sin B . 2019;9(6):1113–44. Available from: http://dx.doi.org/10.1016/j.apsb.2019.10.001
17. Cheung C, Gonzalez FJ. Humanized mouse lines and their application for prediction of human drug metabolism and toxicological risk assessment. J Pharmacol Exp Ther. 2008;327(2):288–99. Available from: http://dx.doi.org/10.1124/jpet.108.141242
18. Lister MF, Sharkey J, Sawatzky DA, Hodgkiss JP, Davidson DJ, Rossi AG, et al. The role of the purinergic P2X7 receptor in inflammation. J Inflamm (Lond) . 2007;4(1):5. Available from: http://dx.doi.org/10.1186/1476-9255-4-5
19. Bunnage ME. Getting pharmaceutical R&D back on target. Nat Chem Biol. 2011;7(6):335–9. Available from: http://dx.doi.org/10.1038/nchembio.581
20. Hay M, Thomas DW, Craighead JL, Economides C, Rosenthal J. Clinical development success rates for investigational drugs. Nat Biotechnol. 2014;32(1):40–51. Available from: http://dx.doi.org/10.1038/nbt.2786
21. Jentzsch V, Osipenko L, Scannell JW, Hickman JA. Costs and causes of oncology drug attrition with the example of insulin-like growth factor-1 receptor inhibitors. JAMA Netw Open. 2023;6(7):e2324977. Available from: http://dx.doi.org/10.1001/jamanetworkopen.2023.24977
22. Navarro-Serna S, Vilarino M, Park I, Gadea J, Ross PJ. Livestock gene editing by one-step embryo manipulation. J Equine Vet Sci. 2020;89(103025):103025. Available from: http://dx.doi.org/10.1016/j.jevs.2020.103025
23. Hart T, Chandrashekhar M, Aregger M, Steinhart Z, Brown KR, MacLeod G, et al. High-resolution CRISPR screens reveal fitness genes and genotype-specific cancer liabilities. Cell. 2015;163(6):1515–26. Available from: http://dx.doi.org/10.1016/j.cell.2015.11.015
24. Ancos-Pintado R, Bragado-García I, Morales ML, García-Vicente R, Arroyo-Barea A, Rodríguez-García A, et al. High-throughput CRISPR screening in hematological neoplasms. Cancers (Basel). 2022;14(15):3612. Available from: http://dx.doi.org/10.3390/cancers14153612
25. Shi J, Wang E, Milazzo JP, Wang Z, Kinney JB, Vakoc CR. Discovery of cancer drug targets by CRISPR-Cas9 screening of protein domains. Nat Biotechnol. 2015;33(6):661–7. Available from: http://dx.doi.org/10.1038/nbt.3235
26. Ormandy EH, Dale J, Griffin G. Genetic engineering of animals: ethical issues, including welfare concerns. Can Vet J. 2011;52(5):544–50. Available from: PMC3078015
27. Tannenbaum J, Bennett BT. Russell and Burch's 3Rs then and now: the need for clarity in definition and purpose. J Am Assoc Lab Anim Sci. 2015;54(2):120–32. Available from: https://pmc.ncbi.nlm.nih.

gov/articles/PMC4382615/
28. Shen MM, Abate-Shen C. Molecular genetics of prostate cancer: new prospects for old challenges. Genes Dev . 2010;24(18):1967–2000. Available from: http://dx.doi.org/10.1101/gad.1965810
29. Buhmeida A, Pyrhönen S, Laato M, Collan Y. Prognostic factors in prostate cancer. Diagn Pathol. 2006;1(1):4. Available from: http://dx.doi.org/10.1186/1746-1596-1-4
30. Gingrich JR, Barrios RJ, Morton RA, Boyce BF, DeMayo FJ, Finegold MJ, et al. Metastatic prostate cancer in a transgenic mouse. Cancer Res. 1996;56(18):4096–102. Available from: 8797572
31. Thomsen MK, Busk M. Pre-clinical models to study human prostate cancer. Preprints. 2023. Available from: http://dx.doi.org/10.20944/preprints202307.1424.v1
32. Testa U, Castelli G, Pelosi E. Cellular and molecular mechanisms underlying prostate cancer development: Therapeutic implications. Medicines (Basel). 2019;6(3):82. Available from: http://dx.doi.org/10.3390/medicines6030082
33. Thummel C, Tjian R, Grodzicker T. Expression of SV40 T antigen under control of adenovirus promoters. Cell. 1981;23(3):825–36. Available from: http://dx.doi.org/10.1016/0092-8674(81)90447-5
34. Cristofano D. Pten is essential for embryonic development and tumour suppression. Nat Genet. 1998;19(4):348–55. Available from: https://doi.org/10.1038/1235
35. Suzuki A, de la Pompa JL, Stambolic V, Elia AJ, Sasaki T, del Barco Barrantes I, et al. High cancer susceptibility and embryonic lethality associated with mutation of the PTEN tumor suppressor gene in mice. Curr Biol. 1998;8(21):1169–78. Available from: http://dx.doi.org/10.1016/s0960-9822(07)00488-5
36. Carracedo A, Alimonti A, Pandolfi PP. PTEN level in tumor suppression: how much is too little? Cancer Res. 2011;71(3):629–33. Available from: http://dx.doi.org/10.1158/0008-5472.CAN-10-2488
37. Aquila S, Santoro M, Caputo A, Panno ML, Pezzi V, De Amicis F. The tumor suppressor PTEN as molecular switch node regulating cell metabolism and autophagy: Implications in immune system and tumor microenvironment. Cells. 2020;9(7):1725. Available from: http://dx.doi.org/10.3390/cells9071725
38. Kim MJ, Cardiff RD, Desai N, Banach-Petrosky WA, Parsons R, Shen MM, et al. Cooperativity of Nkx3.1 and Pten loss of function in a mouse model of prostate carcinogenesis. Proc Natl Acad Sci U S A. 2002;99(5):2884–9. Available from: http://dx.doi.org/10.1073/pnas.042688999
39. Antao AM, Ramakrishna S, Kim K-S. The role of Nkx3.1 in cancers and stemness. Int J Stem Cells. 2021;14(2):168–79. Available from: http://dx.doi.org/10.15283/ijsc20121
40. Kratzer TB, Jemal A, Miller KD, Nash S, Wiggins C, Redwood D, et al. Cancer statistics for American Indian and Alaska Native individuals, 2022: Including increasing disparities in early onset colorectal cancer. CA Cancer J Clin. 2023;73(2):120–46. Available from: http://dx.doi.org/10.3322/caac.21757
41. Metzger-Filho O, Sun Z, Viale G, Price KN, Crivellari D, Snyder RD, et al. Patterns of Recurrence and outcome according to breast cancer subtypes in lymph node-negative disease: results from international breast cancer study group trials VIII and IX. J Clin Oncol. 2013;31(25):3083–90. Available from: http://dx.doi.org/10.1200/JCO.2012.46.1574
42. Liu C-L, Huang W-C, Cheng S-P, Chen M-J, Lin C-H, Chang S-C, et al. Characterization of mammary tumors arising from MMTV-PyVT transgenic mice. Curr Issues Mol Biol. 2023;45(6):4518–28. Available from: http://dx.doi.org/10.3390/cimb45060286
43. Lin EY, Jones JG, Li P, Zhu L, Whitney KD, Muller WJ, et al. Progression to malignancy in the polyoma middle T oncoprotein mouse breast cancer model provides a reliable model for human diseases. Am J Pathol. 2003;163(5):2113–26. Available from: http://dx.doi.org/10.1016/S0002-9440(10)63568-7
44. Regua AT, Arrigo A, Doheny D, Wong GL, Lo H-W. Transgenic mouse models of breast cancer. Cancer Lett. 2021;516:73–83. Available from: http://dx.doi.org/10.1016/j.canlet.2021.05.027
45. Shi Y, Massagué J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. Cell. 2003;113(6):685–700. Available from: http://dx.doi.org/10.1016/s0092-8674(03)00432-x
46. Siegel PM, Massagué J. Cytostatic and apoptotic actions of TGF-beta in homeostasis and cancer. Nat Rev Cancer. 2003;3(11):807–21. Available from: http://dx.doi.org/10.1038/nrc1208
47. Zhu Q, Maitra U, Johnston D, Lozano M, Dudley JP. The homeodomain protein CDP regulates mammary-specific gene transcription and tumorigenesis. Mol Cell Biol. 2004;24(11):4810–23. Available from: http://dx.doi.org/10.1128/MCB.24.11.4810-4823.2004
48. Mink S, Härtig E, Jennewein P, Doppler W, Cato AC. A mammary cell-specific enhancer in mouse mammary tumor virus DNA is composed of multiple regulatory elements including binding sites for CTF/NFI and a novel transcription factor, mammary cell-activating factor. Mol Cell Biol. 1992;12(11):4906–18. Available from: http://dx.doi.org/10.1128/mcb.12.11.4906-4918.1992
49. Rauner G. Using organoids to tap mammary gland diversity for novel insight. J Mammary Gland Biol Neoplasia. 2024;29(1):7. Available from: http://dx.doi.org/10.1007/s10911-024-09559-z
50. Katoh M, Katoh M. Precision medicine for human cancers with Notch signaling dysregulation (Review). Int J Mol Med. 2020;45(2):279–97. Available from: http://dx.doi.org/10.3892/ijmm.2019.4418
51. Ross SR. Mouse mammary tumor virus molecular biology and oncogenesis. Viruses. 2010;2(9):2000–12. Available from: http://dx.doi.org/10.3390/v2092000
52. Smith JA, Barraclough R, Fernig DG, Rudland PS. Identification of alpha transforming growth factor as a possible local trophic agent for the mammary gland. J Cell Physiol. 1989;141(2):362–70. Available from: http://dx.doi.org/10.1002/jcp.1041410218
53. Normanno N, Ciardiello F, Brandt R, Salomon DS. Epidermal growth factor-related peptides in the pathogenesis of human breast cancer. Breast Cancer Res Treat. 1994;29(1):11–27. Available from: http://dx.doi.org/10.1007/bf00666178
54. Gasco M, Shami S, Crook T. The p53 pathway in breast cancer. Breast Cancer Res. 2002;4(2):70–6. Available from: http://dx.doi.org/10.1186/bcr426
55. Jacks T, Remington L, Williams BO, Schmitt EM, Halachmi S, Bronson RT, et al. Tumor spectrum analysis in p53-mutant mice. Curr Biol. 1994;4(1):1–7. Available from: http://dx.doi.org/10.1016/s0960-9822(00)00002-6
56. Harvey M, McArthur MJ, Montgomery CA Jr, Butel JS, Bradley A, Donehower LA. Spontaneous and carcinogen-induced tumorigenesis in p53-deficient mice. Nat Genet. 1993;5(3):225–9. Available from: http://dx.doi.org/10.1038/ng1193-225
57. Engel BE, Cress WD, Santiago-Cardona PG. The retinoblastoma protein: A master tumor suppressor acts as a link between cell cycle and cell adhesion. Cell Health Cytoskelet. 2015;7:1–10. Available from: http://dx.doi.org/10.2147/CHC.S28079
58. Herschkowitz JI, He X, Fan C, Perou CM. The functional loss of the retinoblastoma tumour suppressor is a common event in basal-like and luminal B breast carcinomas. Breast Cancer Res. 2008;10(5):R75. Available from: http://dx.doi.org/10.1186/bcr2142
59. Witkiewicz AK, Knudsen ES. Retinoblastoma tumor suppressor pathway in breast cancer: prognosis, precision medicine, and therapeutic interventions. Breast Cancer Res. 2014;16(3):207. Available from: http://dx.doi.org/10.1186/bcr3652
60. Kanno A, Masamune A, Hanada K, Kikuyama M, Kitano M. Advances in Early Detection of Pancreatic Cancer. Diagnostics (Basel) . 2019;9(1):18. Available from: http://dx.doi.org/10.3390/diagnostics9010018
61. Ferri-Borgogno S, Barui S, McGee AM, Griffiths T, Singh PK, Piett CG, et al. Paradoxical role of AT-rich interactive domain 1A in restraining pancreatic carcinogenesis. Cancers (Basel). 2020;12(9):2695. Available from: http://dx.doi.org/10.3390/cancers12092695
62. Kimura Y, Fukuda A, Ogawa S, Maruno T, Takada Y, Tsuda M, et al. ARID1A maintains differentiation of pancreatic ductal cells and inhibits development of pancreatic ductal adenocarcinoma in mice. Gastroenterology. 2018;155(1):194-209.e2. Available from: http://

dx.doi.org/10.1053/j.gastro.2018.03.039
63. Weng C-C, Lin Y-C, Cheng K-H. The use of genetically engineered mouse models for studying the function of mutated driver genes in pancreatic cancer. J Clin Med. 2019;8(9):1369. Available from: http://dx.doi.org/10.3390/jcm8091369
64. Luo J. KRAS mutation in pancreatic cancer. Semin Oncol. 2021;48(1):10–8. Available from: http://dx.doi.org/10.1053/j.seminoncol.2021.02.003
65. Aguirre AJ, Bardeesy N, Sinha M, Lopez L, Tuveson DA, Horner J, et al. Activated Kras and Ink4a/Arf deficiency cooperate to produce metastatic pancreatic ductal adenocarcinoma. Genes Dev. 2003;17(24):3112–26. Available from: http://dx.doi.org/10.1101/gad.1158703
66. Lewis BC, Klimstra DS, Varmus HE. The c-myc and PyMT oncogenes induce different tumor types in a somatic mouse model for pancreatic cancer. Genes Dev. 2003;17(24):3127–38. Available from: http://dx.doi.org/10.1101/gad.1140403
67. Riggs PK, Rho O, DiGiovanni J. Alteration of Egr-1 mRNA during multistage carcinogenesis in mouse skin. Mol Carcinog. 2000;27(4):247–51. Available from: http://dx.doi.org/10.1002/(sici)1098-2744(200004)27:4<247::aid-mc1>3.0.co;2-4
68. Abel EL, Angel JM, Kiguchi K, DiGiovanni J. Multi-stage chemical carcinogenesis in mouse skin: fundamentals and applications. Nat Protoc. 2009;4(9):1350–62. Available from: http://dx.doi.org/10.1038/nprot.2009.120
69. Coussens LM, Hanahan D, Arbeit JM. Genetic predisposition and parameters of malignant progression in K14-HPV16 transgenic mice. Am J Pathol. 1996;149(6):1899–917. Available from: PMC1865365
70. Peixoto da Silva S, Santos JMO, Mestre VF, Medeiros-Fonseca B, Oliveira PA, M S M Bastos M, et al. Human Papillomavirus 16-transgenic mice as a model to study cancer-associated cachexia. Int J Mol Sci. 2020;21(14):5020. Available from: http://dx.doi.org/10.3390/ijms21145020
71. Cochicho D, Nunes A, Gomes JP, Martins L, Cunha M, Medeiros-Fonseca B, et al. Characterization of the human Papillomavirus 16 oncogenes in K14HPV16 mice: Sublineage A1 drives multi-organ carcinogenesis. Int J Mol Sci. 2022;23(20):12371. Available from: http://dx.doi.org/10.3390/ijms232012371
72. Zhang X, Bolt M, Guertin MJ, Chen W, Zhang S, Cherrington BD, et al. Peptidylarginine deiminase 2-catalyzed histone H3 arginine 26 citrullination facilitates estrogen receptor α target gene activation. Proc Natl Acad Sci U S A. 2012;109(33):13331–6. Available from: http://dx.doi.org/10.1073/pnas.1203280109
73. Schinko JB, Weber M, Viktorinova I, Kiupakis A, Averof M, Klingler M, et al. Functionality of the GAL4/UAS system in Tribolium requires the use of endogenous core promoters. BMC Dev Biol. 2010;10(1):53. Available from: http://dx.doi.org/10.1186/1471-213X-10-53
74. Zhang T, Dutton-Regester K, Brown KM, Hayward NK. The genomic landscape of cutaneous melanoma. Pigment Cell Melanoma Res. 2016;29(3):266–83. Available from: http://dx.doi.org/10.1111/pcmr.12459
75. Powell MB, Hyman P, Bell OD, Balmain A, Brown K, Alberts D, et al. Hyperpigmentation and melanocytic hyperplasia in transgenic mice expressing the human T24 Ha-ras gene regulated by a mouse tyrosinase promoter. Mol Carcinog. 1995;12(2):82–90. Available from: http://dx.doi.org/10.1002/mc.2940120205
76. Battaglia L, Scomparin A, Dianzani C, Milla P, Muntoni E, Arpicco S, et al. Nanotechnology addressing cutaneous melanoma: The Italian landscape. Pharmaceutics. 2021;13(10):1617. Available from: http://dx.doi.org/10.3390/pharmaceutics13101617
77. Zhang J, Zhao J, Jiang W-J, Shan X-W, Yang X-M, Gao J-G. Conditional gene manipulation: Cre-ating a new biological era. J Zhejiang Univ Sci B. 2012;13(7):511–24. Available from: http://dx.doi.org/10.1631/jzus.B1200042
78. Chen D, Thayer TC, Wen L, Wong FS. Mouse models of autoimmune diabetes: The nonobese diabetic (NOD) mouse. Methods Mol Biol. 2020;2128:87–92. Available from: http://dx.doi.org/10.1007/978-1-0716-0385-7_6
79. Niu L, Xu Y-C, Dai Z, Tang H-Q. Gene therapy for type 1 diabetes mellitus in rats by gastrointestinal administration of chitosan nanoparticles containing human insulin gene. World J Gastroenterol. 2008;14(26):4209–15. Available from: http://dx.doi.org/10.3748/wjg.14.4209
80. Chen H, Charlat O, Tartaglia LA, Woolf EA, Weng X, Ellis SJ, et al. Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice. Cell. 1996;84(3):491–5. Available from: http://dx.doi.org/10.1016/s0092-8674(00)81294-5
81. Suriano F, Vieira-Silva S, Falony G, Roumain M, Paquot A, Pelicaen R, et al. Novel insights into the genetically obese (ob/ob) and diabetic (db/db) mice: two sides of the same coin. Microbiome. 2021;9(1):147. Available from: http://dx.doi.org/10.1186/s40168-021-01097-8
82. Vatashchuk MV, Bayliak MM, Hurza VV, Storey KB, Lushchak VI. Metabolic syndrome: Lessons from rodent and Drosophila models. Biomed Res Int. 2022;2022:5850507. Available from: http://dx.doi.org/10.1155/2022/5850507
83. de Luca C, Kowalski TJ, Zhang Y, Elmquist JK, Lee C, Kilimann MW, et al. Complete rescue of obesity, diabetes, and infertility in db/db mice by neuron-specific LEPR-B transgenes. J Clin Invest. 2005;115(12):3484–93. Available from: http://dx.doi.org/10.1172/JCI24059
84. Tartaglia LA. The leptin receptor. J Biol Chem. 1997;272(10):6093–6. Available from: http://dx.doi.org/10.1074/jbc.272.10.6093
85. Makino S, Kunimoto K, Muraoka Y, Mizushima Y, Katagiri K, Tochino Y. Breeding of a non-obese, diabetic strain of mice. Jikken Dobutsu. 1980;29(1):1–13. Available from: http://dx.doi.org/10.1538/expanim1978.29.1_1
86. Cai X, McGinnis JF. Diabetic retinopathy: Animal models, therapies, and perspectives. J Diabetes Res. 2016;2016:3789217. Available from: http://dx.doi.org/10.1155/2016/3789217
87. Klebig ML, Wilkinson JE, Geisler JG, Woychik RP. Ectopic expression of the agouti gene in transgenic mice causes obesity, features of type II diabetes, and yellow fur. Proc Natl Acad Sci U S A. 1995;92(11):4728–32. Available from: http://dx.doi.org/10.1073/pnas.92.11.4728
88. Pandey S, Dvorakova MC, Pandey S, Dvorakova MC. Future Perspective of Diabetic Animal Models. Endocr Metab Immune Disord Drug Targets. 2020;20(1):25–38. Available from: https://doi.org/10.2174/1871530319666190626143832
89. Deng Y, Scherer PE. Adipokines as novel biomarkers and regulators of the metabolic syndrome: Adipokines as novel biomarkers and regulators of the metabolic syndrome. Ann N Y Acad Sci. 2010;1212(1):E1–19. Available from: http://dx.doi.org/10.1111/j.1749-6632.2010.05875.x
90. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab. 2004;89(6):2548–56. Available from: http://dx.doi.org/10.1210/jc.2004-0395
91. Arioglu E, Rother KI, Reitman ML, Premkumar A, Taylor SI. Lipoatrophy syndromes: when "too little fat" is a clinical problem: Lipoatrophy syndromes. Pediatr Diabetes. 2000;1(3):155–68. Available from: http://dx.doi.org/10.1034/j.1399-5448.2000.010307.x
92. Friedman J. The long road to leptin. J Clin Invest. 2016;126(12):4727–34. Available from: http://dx.doi.org/10.1172/JCI91578
93. Chandrasekaran P, Weiskirchen R. The role of obesity in type 2 diabetes mellitus-an overview. Int J Mol Sci. 2024;25(3):1882. Available from: http://dx.doi.org/10.3390/ijms25031882
94. Reifsnyder PC, Churchill G, Leiter EH. Maternal environment and genotype interact to establish diabesity in mice. Genome Res. 2000;10(10):1568–78. Available from: http://dx.doi.org/10.1101/gr.147000
95. Kim JH, Saxton AM. The TALLYHO mouse as a model of human type 2 diabetes. Methods Mol Biol. 2012;933:75–87. Available from: http://dx.doi.org/10.1007/978-1-62703-068-7_6
96. Parilla JH, Willard JR, Barrow BM, Zraika S. A mouse model of beta-cell dysfunction as seen in human type 2 diabetes. J Diabetes Res. 2018;2018:6106051. Available from: http://dx.doi.org/10.1155/2018/6106051

97. Wauman J, Zabeau L, Tavernier J. The Leptin Receptor Complex: Heavier Than Expected? Front Endocrinol (Lausanne). 2017. Feb 21:8:30. Available from: 10.3389/fendo.2017.00030.
98. Brun P, Castagliuolo I, Di Leo V, Buda A, Pinzani M, Palù G, et al. Increased intestinal permeability in obese mice: new evidence in the pathogenesis of nonalcoholic steatohepatitis. Am J Physiol Gastrointest Liver Physiol. 2007;292(2):G518-25. Available from: http://dx.doi.org/10.1152/ajpgi.00024.2006
99. Geurts L, Lazarevic V, Derrien M, Everard A, Van Roye M, Knauf C, et al. Altered gut microbiota and endocannabinoid system tone in obese and diabetic leptin-resistant mice: impact on apelin regulation in adipose tissue. Front Microbiol. 2011;2:149. Available from: http://dx.doi.org/10.3389/fmicb.2011.00149
100. Coleman DL. Obese and diabetes: two mutant genes causing diabetes-obesity syndromes in mice. Diabetologia. 1978;14(3):141–8. Available from: http://dx.doi.org/10.1007/bf00429772
101. Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, et al. Effects of the obese gene product on body weight regulation in ob/ob mice. Science. 1995;269(5223):540–3. Available from: http://dx.doi.org/10.1126/science.7624776
102. Simon DB, Lifton RP. Mutations in Na(K)Cl transporters in Gitelman's and Bartter's syndromes. Curr Opin Cell Biol. 1998;10(4):450–4. Available from: http://dx.doi.org/10.1016/s0955-0674(98)80057-4
103. Crisan D, Carr J. Angiotensin I-converting enzyme: genotype and disease associations. J Mol Diagn. 2000;2(3):105–15. Available from: http://dx.doi.org/10.1016/S1525-1578(10)60624-1
104. Getz GS, Reardon CA. Do the apoe-/- and Ldlr-/- mice yield the same insight on atherogenesis? Arterioscler Thromb Vasc Biol. 2016;36(9):1734–41. Available from: http://dx.doi.org/10.1161/ATVBAHA.116.306874
105. Zhang SH, Reddick RL, Piedrahita JA, Maeda N. Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. Science. 1992;258(5081):468–71. Available from: http://dx.doi.org/10.1126/science.1411543
106. Ishibashi S, Brown MS, Goldstein JL, Gerard RD, Hammer RE, Herz J. Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus-mediated gene delivery. J Clin Invest. 1993;92(2):883–93. Available from: http://dx.doi.org/10.1172/JCI116663
107. Roselaar SE, Kakkanathu PX, Daugherty A. Lymphocyte populations in atherosclerotic lesions of apoE -/- and LDL receptor -/- mice. Decreasing density with disease progression: Decreasing density with disease progression. Arterioscler Thromb Vasc Biol. 1996;16(8):1013–8. Available from: http://dx.doi.org/10.1161/01.atv.16.8.1013
108. Stylianou IM, Bauer RC, Reilly MP, Rader DJ. Genetic basis of atherosclerosis: insights from mice and humans. Circ Res. 2012;110(2):337–55. Available from: http://dx.doi.org/10.1161/CIRCRESAHA.110.230854
109. Hopkins PN. Molecular biology and genetics of atherosclerosis. In: Preventive Cardiology: Companion to Braunwald's Heart Disease. Elsevier; 2011. p. 86–120.
110. Staršíchová A. SR-B1-/-ApoE-R61h/h mice mimic human coronary heart disease. Cardiovasc Drugs Ther. 2023; Available from: http://dx.doi.org/10.1007/s10557-023-07475-8
111. Getz GS, Reardon CA. Insights from Murine studies on the site specificity of atherosclerosis. Int J Mol Sci. 2024;25(12):6375. Available from: http://dx.doi.org/10.3390/ijms25126375
112. Miao L-N, Pan D, Shi J, Du J-P, Chen P-F, Gao J, et al. Role and mechanism of PKC-δ for cardiovascular disease: Current status and perspective. Front Cardiovasc Med. 2022;9:816369. Available from: http://dx.doi.org/10.3389/fcvm.2022.816369
113. Jones LR, Suzuki YJ, Wang W, Kobayashi YM, Ramesh V, Franzini-Armstrong C, et al. Regulation of Ca2+ signaling in transgenic mouse cardiac myocytes overexpressing calsequestrin. J Clin Invest. 1998;101(7):1385–93. Available from: http://dx.doi.org/10.1172/JCI1362
114. Bisping E, Ikeda S, Kong SW, Tarnavski O, Bodyak N, McMullen JR, et al. Gata4 is required for maintenance of postnatal cardiac function and protection from pressure overload-induced heart failure. Proc Natl Acad Sci U S A. 2006;103(39):14471–6. Available from: http://dx.doi.org/10.1073/pnas.0602543103
115. Wilson RJ, Drake JC, Cui D, Zhang M, Perry HM, Kashatus JA, et al. Conditional MitoTimer reporter mice for assessment of mitochondrial structure, oxidative stress, and mitophagy. Mitochondrion. 2019;44:20–6. Available from: http://dx.doi.org/10.1016/j.mito.2017.12.008
116. Kawaguchi A, Miyata T, Sawamoto K, Takashita N, Murayama A, Akamatsu W, et al. Nestin-EGFP transgenic mice: visualization of the self-renewal and multipotency of CNS stem cells. Mol Cell Neurosci. 2001;17(2):259–73. Available from: http://dx.doi.org/10.1006/mcne.2000.0925
117. Chen W, Hu Y, Ju D. Gene therapy for neurodegenerative disorders: advances, insights and prospects. Acta Pharm Sin B. 2020;10(8):1347–59. Available from: http://dx.doi.org/10.1016/j.apsb.2020.01.015
118. Lee JH, Wang J-H, Chen J, Li F, Edwards TL, Hewitt AW, et al. Gene therapy for visual loss: Opportunities and concerns. Prog Retin Eye Res. 2019;68:31–53. Available from: http://dx.doi.org/10.1016/j.preteyeres.2018.08.003
119. Ehmke T, Leckelt J, Reichard M, Weiss H, Hovakimyan M, Heisterkamp A, et al. In vivo nonlinear imaging of corneal structures with special focus on BALB/c and streptozotocin-diabetic Thy1-YFP mice. Exp Eye Res. 2016;146:137–44. Available from: http://dx.doi.org/10.1016/j.exer.2015.11.024
120. Vetrivel KS, Zhang Y-W, Xu H, Thinakaran G. Pathological and physiological functions of presenilins. Mol Neurodegener. 2006;1(1):4. Available from: http://dx.doi.org/10.1186/1750-1326-1-4
121. Zhu Y-L, Sun M-F, Jia X-B, Cheng K, Xu Y-D, Zhou Z-L, et al. Neuroprotective effects of Astilbin on MPTP-induced Parkinson's disease mice: Glial reaction, α-synuclein expression and oxidative stress. Int Immunopharmacol. 2019;66:19–27. Available from: http://dx.doi.org/10.1016/j.intimp.2018.11.004
122. Liu C, Xie W, Gui C, Du Y. Pronuclear microinjection and oviduct transfer procedures for transgenic mouse production. Methods Mol Biol. 2013;1027:217–32. Available from: http://dx.doi.org/10.1007/978-1-60327-369-5_10
123. Ballatore C, Lee VM-Y, Trojanowski JQ. Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. Nat Rev Neurosci. 2007;8(9):663–72. Available from: http://dx.doi.org/10.1038/nrn2194
124. Clavaguera F, Bolmont T, Crowther RA, Abramowski D, Frank S, Probst A, et al. Transmission and spreading of tauopathy in transgenic mouse brain. Nat Cell Biol. 2009;11(7):909–13. Available from: http://dx.doi.org/10.1038/ncb1901
125. Kaufman SK, Sanders DW, Thomas TL, Ruchinskas AJ, Vaquer-Alicea J, Sharma AM, et al. Tau prion strains dictate patterns of cell pathology, progression rate, and regional vulnerability in vivo. Neuron. 2016;92(4):796–812. Available from: http://dx.doi.org/10.1016/j.neuron.2016.09.055
126. Clavaguera F, Akatsu H, Fraser G, Crowther RA, Frank S, Hench J, et al. Brain homogenates from human tauopathies induce tau inclusions in mouse brain. Proc Natl Acad Sci U S A. 2013;110(23):9535–40. Available from: http://dx.doi.org/10.1073/pnas.1301175110
127. Gotz J. PL-03-03: Animal models for Alzheimer's disease and frontotemporal dementia. Alzheimers Dement. 2009;5(4S_Part_4):P118–P118. Available from: http://dx.doi.org/10.1016/j.jalz.2009.05.372
128. Gouras GK, Tsai J, Naslund J, Vincent B, Edgar M, Checler F, et al. Intraneuronal Abeta42 accumulation in human brain. Am J Pathol. 2000;156(1):15–20. Available from: http://dx.doi.org/10.1016/s0002-9440(10)64700-1
129. Gouras GK, Tampellini D, Takahashi RH, Capetillo-Zarate E. Intraneuronal beta-amyloid accumulation and synapse pathology in Alzheimer's disease. Acta Neuropathol. 2010;119(5):523–41.

Available from: http://dx.doi.org/10.1007/s00401-010-0679-9
130. Peggion C, Scalcon V, Massimino ML, Nies K, Lopreiato R, Rigobello MP, et al. SOD1 in ALS: Taking stock in pathogenic mechanisms and the role of glial and muscle cells. Antioxidants (Basel). 2022;11(4):614. Available from: http://dx.doi.org/10.3390/antiox11040614
131. Jaarsma D, Haasdijk ED, Grashorn JA, Hawkins R, van Duijn W, Verspaget HW, et al. Human Cu/Zn superoxide dismutase (SOD1) overexpression in mice causes mitochondrial vacuolization, axonal degeneration, and premature motoneuron death and accelerates motoneuron disease in mice expressing a familial amyotrophic lateral sclerosis mutant SOD1. Neurobiol Dis. 2000;7(6 Pt B):623–43. Available from: http://dx.doi.org/10.1006/nbdi.2000.0299
132. Jonsson PA, Graffmo KS, Andersen PM, Brännström T, Lindberg M, Oliveberg M, et al. Disulphide-reduced superoxide dismutase-1 in CNS of transgenic amyotrophic lateral sclerosis models. Brain. 2006;129(Pt 2):451–64. Available from: http://dx.doi.org/10.1093/brain/awh704
133. Baccili Cury Megid T, Farooq AR, Wang X, Elimova E. Gastric cancer: Molecular mechanisms, novel targets, and immunotherapies: From bench to clinical therapeutics. Cancers (Basel). 2023;15(20):5075. Available from: http://dx.doi.org/10.3390/cancers15205075
134. Zhang Q, Yang M, Zhang P, Wu B, Wei X, Li S. Deciphering gastric inflammation-induced tumorigenesis through multi-omics data and AI methods. Cancer Biol Med. 2023;21(4). Available from: http://dx.doi.org/10.20892/j.issn.2095-3941.2023.0129
135. Fox JG, Rogers AB, Whary MT, Ge Z, Ohtani M, Jones EK, et al. Accelerated progression of gastritis to dysplasia in the pyloric antrum of TFF2 -/- C57BL6 x Sv129 Helicobacter pylori-infected mice. Am J Pathol. 2007;171(5):1520–8. Available from: http://dx.doi.org/10.2353/ajpath.2007.070249
136. Halberg RB, Chen X, Amos-Landgraf JM, White A, Rasmussen K, Clipson L, et al. The pleiotropic phenotype of Apc mutations in the mouse: allele specificity and effects of the genetic background. Genetics. 2008;180(1):601–9. Available from: http://dx.doi.org/10.1534/genetics.108.091967
137. Aoki K, Taketo MM. Adenomatous polyposis coli (APC): a multi-functional tumor suppressor gene. J Cell Sci. 2007;120(Pt 19):3327–35. Available from: http://dx.doi.org/10.1242/jcs.03485
138. Oshima M, Dinchuk JE, Kargman SL, Oshima H, Hancock B, Kwong E, et al. Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell. 1996;87(5):803–9. Available from: http://dx.doi.org/10.1016/s0092-8674(00)81988-1
139. Xue Y, Johnson R, Desmet M, Snyder PW, Fleet JC. Generation of a transgenic mouse for colorectal cancer research with intestinal cre expression limited to the large intestine. Mol Cancer Res. 2010;8(8):1095–104. Available from: http://dx.doi.org/10.1158/1541-7786.MCR-10-0195
140. Colnot S, Niwa-Kawakita M, Hamard G, Godard C, Le Plenier S, Houbron C, et al. Colorectal cancers in a new mouse model of familial adenomatous polyposis: influence of genetic and environmental modifiers. Lab Invest. 2004;84(12):1619–30. Available from: http://dx.doi.org/10.1038/labinvest.3700180
141. Fleet JC, Wood RJ. Specific 1,25(OH)2D3-mediated regulation of transcellular calcium transport in Caco-2 cells. Am J Physiol. 1999;276(4):G958-64. Available from: http://dx.doi.org/10.1152/ajpgi.1999.276.4.G958
142. Niu Z-S, Li B-K, Wang M. Expression of p53 and C-myc genes and its clinical relevance in the hepatocellular carcinomatous and pericarcinomatous tissues. World J Gastroenterol. 2002;8(5):822–6. Available from: http://dx.doi.org/10.3748/wjg.v8.i5.822
143. Xu C, Xu Z, Zhang Y, Evert M, Calvisi DF, Chen X. β-Catenin signaling in hepatocellular carcinoma. J Clin Invest. 2022;132(4). Available from: http://dx.doi.org/10.1172/JCI154515
144. Kumar TR, Larson M, Wang H, McDermott J, Bronshteyn I. Transgenic mouse technology: principles and methods. Methods Mol Biol. 2009;590:335–62. Available from: http://dx.doi.org/10.1007/978-1-60327-378-7_22
145. Brown SDM. Advances in mouse genetics for the study of human disease. Hum Mol Genet. 2021;30(R2):R274–84. Available from: http://dx.doi.org/10.1093/hmg/ddab153

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