BIOMICE

AN ODE TO ANIMAL MODES

BY:-

Vartika singh

Swati Kumari

Rohan Irlapalle Reddy

Sandesh Jadhav

EDITOR:-

INTRODUCTION

What is animal research?

Scientific research using animals is vital to our continued and improved understanding of human and animal health. Animals are used to help us understand living organisms, study disease, and develop and test new medical treatments.

Animal research is also used to keep people, animals and the environment safe from new medicines and chemicals

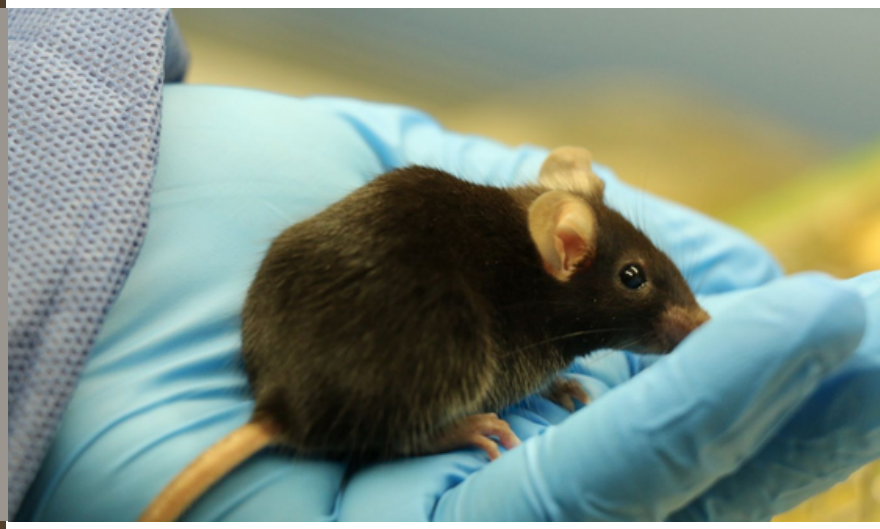
Why use the mouse in research?

- **Over the past century, the house mouse (Mus musculus) has become the preferred mammalian model for genetic research because mouse genome is very similar to our own, making it particularly useful for the study of human diseases.**
- **As a scientific tool, mice have helped to speed up the progress of research and enabled the development of important new drugs.**

The laboratory mouse - a life for research

The laboratory mice used in science are all descendants of the house mouse,Mus musculus.The Jackson laboratory founded by Clarence Cook Little, who produced the inbred mouse strain C57BL It is the primary source for common mouse strains like BALB/c mouse strains, and rat strains, and less common strains like FvB/NJ, and X1/SvJ.

Wild mice and laboratory mice differ in appearance especially in colour. The wild forms usually have a brown coat, whereas albino laboratory strains have white fur and other strains have black fur. Mice have an accelerated lifespan, with one mouse year equaling about 30 human years. Therefore, their entire life cycle can be studied within only two or three years.



-Swati Kumari

A history of mice in scientific research

Where there are people, there are mice

The first record of animals being used as models for human anatomy and physiology dates back some 2,400 years to ancient Greece. Scientists often turn to mice as their first choice if they need to study a mammalian model. Mice share around 80% of our genes, they are close to us on the evolutionary tree and are physiologically similar to humans. They are small and have a short lifespan, breed rapidly and do well in captivity.

The First Mouse Model

Mouse-fancier Miss Abbie Lathrop raised and sold mice and was frequently presented as an eccentric hobbyist who had an unusual attraction to mice. Her deliberate and precise mouse breeding produced one of the earliest accepted scientific creatures. Her mice were the first to be utilized in a laboratory setting for genetic study in 1902, and some are still in use today.Mammalian geneticist William E. Castle purchased some of Lathrop's mice in 1902, he trained Clarence Cook Little, and he was credited with creating the modern lab mouse in 1909. The first inbred strain of mice was developed by CC Little, who later founded the Jackson Laboratory.

Dogs might well be man's best friend, but Mus musculus is our greatest ally.

Abbie E. C. Lathrop (1868 – 1918) was a rodent fancier who bred fancy mice and inbred strains for animal models, particularly for research on development and hereditary properties of cancer.She bred Japanese waltzing mice as well as fancy mice.The mice had straw bedding and lived in wooden boxes. They were fed a diet of oats and crackers.

-Swati Kumari

Types of Mouse Models in Research

Genetically modified mouse/genetically engineered mouse model (GEMM)

is a mouse (Mus musculus) that has had its genome altered through the use of genetic engineering techniques. Genetically modified mice are commonly used for research or as animal models of human diseases, and are also used for research on genes.ogether with patient-derived xenografts (PDXs), GEMMs are the most common in vivo models in cancer research. GEMMs are also of great interest for drug development, as they facilitate target validation and the study of response, resistance, toxicity and pharmacodynamics.

Knock out mice is an example of GEMM in which existing gene is inactivated by replacing it or disrupting it with an artificial piece of DNA.Example- p53 knockout mouse .

Hybrid Mice

Hybrid mice and rats are the offspring of two dissimilar parents and tend to be more robust than the parental strains, with longer lifespan and fewer, or lower incidence of, strain-specific pathologies.As long as the parental strains exist, they can be repeatedly produced. Example- B6C3 F1 hybrid mice .

Immunodeficient Mice

Immunodeficient mice refer to the mice with defects in one or more immune components (such as T, B, NK cells) in the immune system. This type of mouse is widely used in the research of oncology (tumor growth, metastasis, anti-tumor drug screening), immunology (mechanisms of immune cell development and proliferation, pathology of immune diseases), infectious diseases (pathogenic mechanisms of viral/bacterial infectious diseases), and stem cell biology (human stem cell transplantation), and more. Examples- BALB/c nude Mice, NOD SCID Mouse, C-NKG Mice.

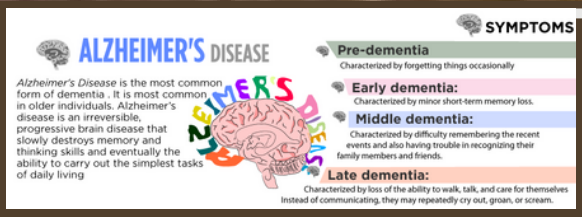
Humanized Mice

Humanized mouse is a general term that refers to a mouse that has been engrafted with something from a human. This could be a short strand of human DNA, human tissue, a human tumor, a humanized immune system, or parts of the human microbiome

- Scientists can take a tumor from a patient, cut it into pieces, and put these pieces into multiple mice.
- Each fragment then grows and can then be divided into pieces and put into additional mice. Through this process, dozens of humanized mice are created, each with tumors nearly identical to each other and to the original human patient's tumor.
- Scientists can treat different groups of PDX mice with alternative drugs, drug combinations, and drug sequences allowing them to determine which approach works best to eliminate a very specific tumor type.

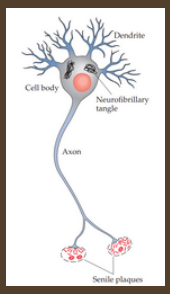
-Swati Kumari

ALZHEIMERS DISEASE



Genetics of AD

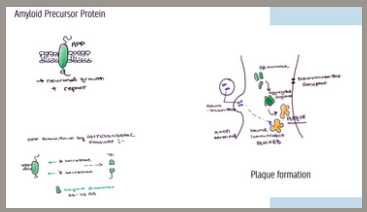
- Mutations in three genes cause autosomal dominant AD: amyloid precursor protein (APP) and the presenilin genes (PSEN1 and PSEN2).



- Inside the neuron of AD patient, it seen that Amyloid Beta is produced and broken down in the Brain.
- The generation of amyloid begins with the protein known as Amyloid Precursor Protein found in the neuronal membrane.
- APP is believed to be essential for neuron growth and repair after injury and like all protein s in the body APP eventually wears out and need to be disposed or recycled.

APP broken down through AMYLOIDGENIC pathway:-

- It produces Amyloid beta , the enzyme BETA Secretase and Gamma Secretase which sequentially cleave APP into fragments resulting in the production of amyloid beta monomers which are proteins made up of 36 to 43 aa.
- Due to increased production of amyloid beta and decrease breakdown of the same. The Excess beta monomers aggregate together forming Plaques.

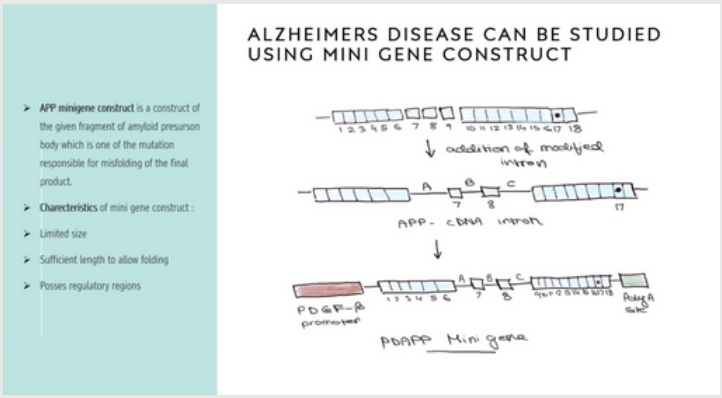


Mouse Model for ALZHEIMERS DISEASE.

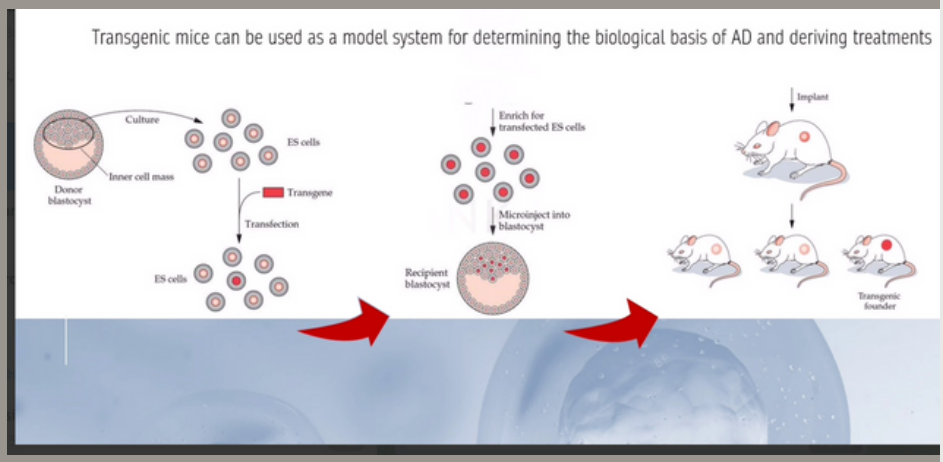
Mouse model for AD were created with transgene that contain mutations in the APP gene that occur in some families with high incidence of early onset of AD.

For this construct:-

- A transgene with APP-717 mutation was constructed from an APP cDNA.
- The exon pf APP cDNA are marked by no. 1 to 18.
- Modified introns added between Exon 6 and 7, 7 and 8, 8 and 9 of the APP cDNA.
- The "APP cDNA-intron " construct is controlled by the Platelet-derived growth factor beta promoter that is expressed in Brain Tissue.
- The complete construct is called the PDAPP mini gene.
- Multiple copies of the PDAPPP mini gene display amyloid plaques, neural death and memory defects.



Establishing Transgenic Mice with APP Gene.

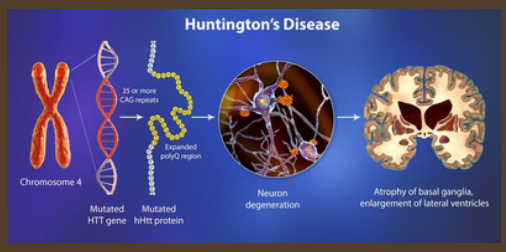


-Vartika Singh

HUNTINGTON'S DISEASE

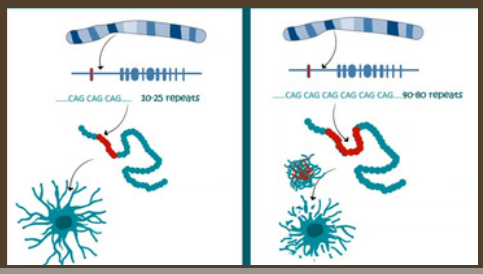
Genetics Of HD

- Huntigton is an autosomal dominant neurological disorder caused by mutation in HTT gene.
- It is characterized by progressive motor, cognitive and behavioral impairment.



- At the genetic level, the alteration that is responsible for Huntington disease is the addition of CAG units to an existing sequential array of these trinucleotides in exon 1 of the HD gene which encodes the huntingtin protein.

- A CAG trinucleotide is the codon for glutamine, and during translation, a contiguous set of these codons produces a string of glutamine residues (polyglutamine) in the huntingtin protein.

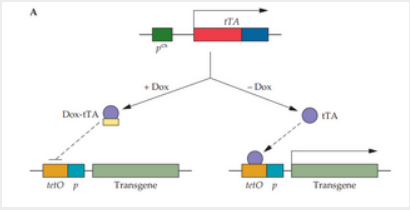


A Transgenic Mouse Model of Huntington Disease

- Various protocols have been devised for turning on and off the expression of a transgene in a specific cell type at will. Of these methods, the tetracycline-inducible system has been used extensively.

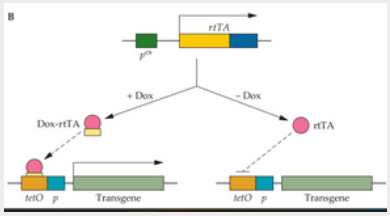
- **Conditional Regulation of Transgene Expression.**

Tet-Off



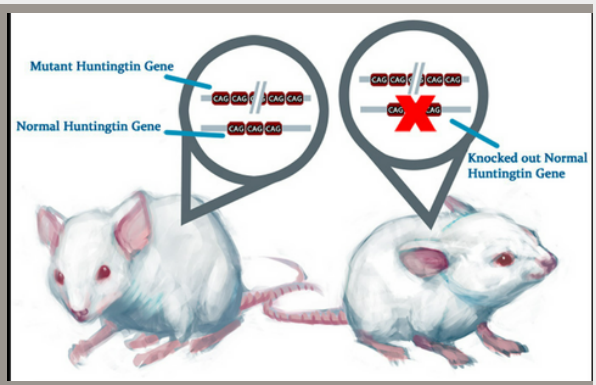
The tTA gene is driven by a cell-specific promoter (pcs) and encodes a tetracycline repressor sequence (red) and a transcription activator (blue). The product of the tTA gene is a protein (tTA [purple circle]) that binds to a tetracy cline operator (tetO)–eukaryotic promoter (p) sequence and, in the absence of doxy cycline (– Dox), activates the transcription of the transgene. When doxycycline is present (+ Dox), it (yellow rectangle) binds to tTA, and the Dox–tTA complex cannot bind to the tetO–promoter region, so the transgene is not transcribed.

Tet-On



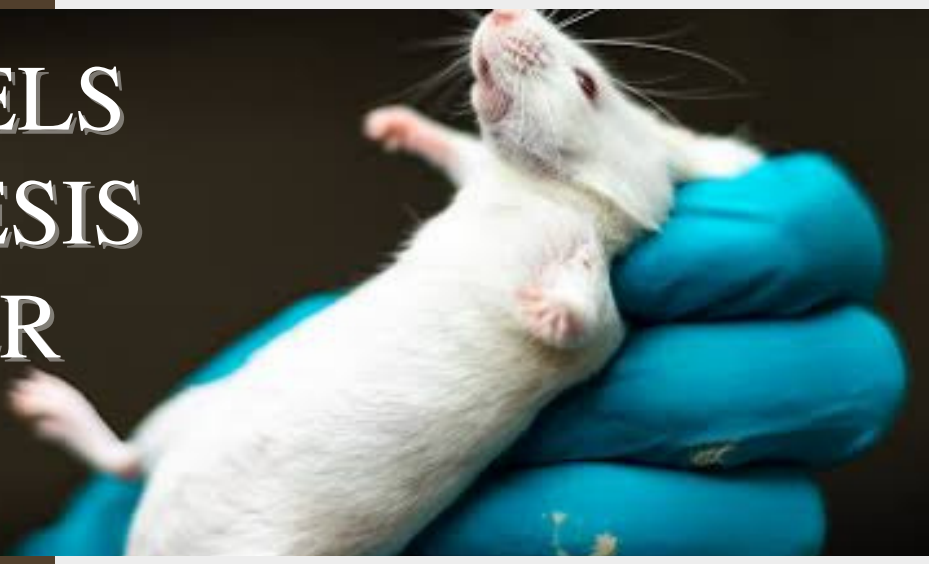
The rtTA gene is driven by a cell-specific promoter (pcs) and encodes a mutated tetracycline repressor sequence (dark yellow) and a transcrip tion activator (blue). The product of the rtTA gene is a protein (rtTA [pink circle]) that does not bind to a tetracycline operator (tetO)–eukaryotic promoter (p) sequence, and in the absence of doxycycline (– Dox), the transgene is not tran scribed. When doxycycline is present (+ Dox), it (yellow rectangle) binds to rtTA, and the Dox–rtTA complex attaches to the tetO–promoter region and initiates the transcription of the transgene.

- **To create a mouse model for Huntington disease, the tet-off system was used with a variant of the HD gene that consists only of exon 1 with 94 CAG repeats as the transgene.**
- **The tTA gene was placed under the control of a promoter that is active in the cells of the forebrain.**
- **. Loss of embryos was avoided during pregnancy by adding doxycycline to the drinking water, which turned off the expression of the mutant HD gene.**
- **A neurological condition that was similar to Huntington disease in humans developed over time in these mice.**
- **Thus, at least in this model, continuous expression of a mutant HD gene is required for establishment of the disease, and brain cells can recover when this synthesis ceases.**



-Vartika Singh

MOUSE MODELS IN ONCOGENESIS AND CANCER THERAPY



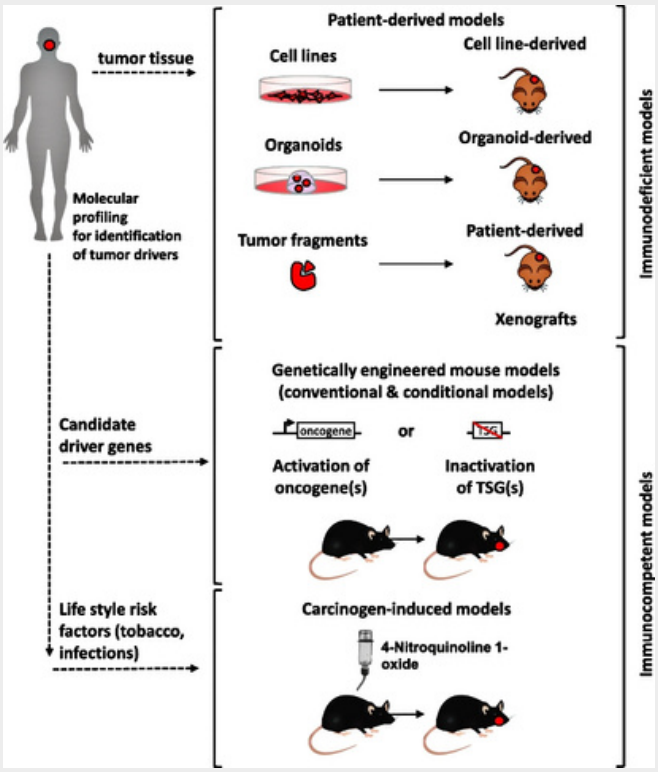
WHAT IS ONCO MOUSE?

Researchers at Harvard Medical School in the early 1980s produced a genetically modified mouse that was highly susceptible to cancer, by introducing an oncogene that can trigger the growth of tumors. The oncomouse (from the Greek word for tumor) was conceived as a valuable means of furthering cancer research. A novel mouse model demonstrates that oncogenic melanocyte stem cells engender melanoma resembling human disease

EVOLUTION OF MOUSE CANCER MODELING:

Since the advent of tumor transplantation models in nude mice 50 years ago, advances in mouse genome engineering have led to the generation of various types of transplantation-based and genetically engineered tumor models to study cancer biology (Fig 2, Table 1). Here, we give an overview of mouse models that are most commonly used in cancer research.

MODELING CANCER IN MICE ONCOGENE:



The laboratory mouse is one of the most powerful tools for both gene discovery and validation in cancer genetics. Recent technologies advances in engineering the mouse genome with chromosome translocations, latent alleles, and tissue-specific and temporally regulated mutations have provided more exacting models of human disease. The marriage of mouse tumor models with rapidly evolving methods to profile genetic and epigenetic alterations in tumors, and to finely map genetic modifier loci, will continue to provide insight into the key pathways leading to tumorigenesis. These discoveries hold great promise for identifying relevant drug targets for treating human cancer.

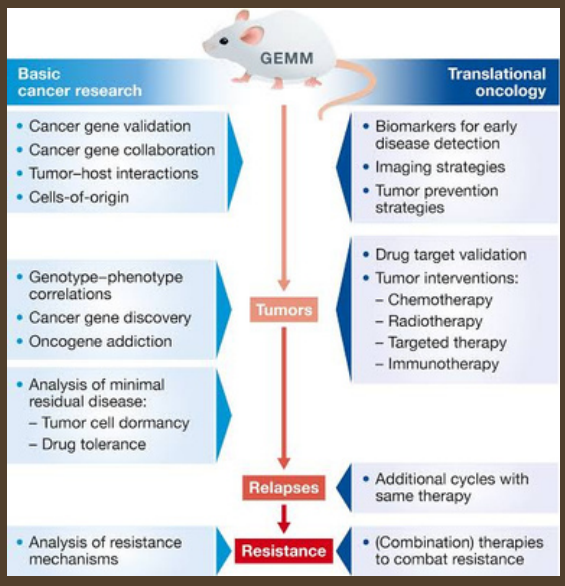
-SANDESH JADHAV

GENETICALLY ENGINEERED MOUSE MODELS IN ONCOLOGY RESEARCH AND CANCER MEDICINE:

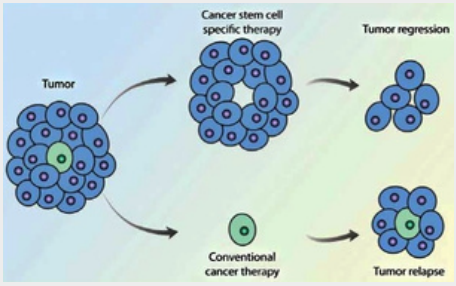
Genetically engineered mouse models (GEMMs) have contributed significantly to the field of cancer research. In contrast to cancer cell inoculation models, GEMMs develop de novo tumors in a natural immune-proficient microenvironment.

As such, GEMMs are generally superior to cancer cell inoculation models, which show no or limited heterogeneity and are often metastatic from the start. Given that

GEMMs capture both tumor cell-intrinsic and cell-extrinsic factors that drive de novo tumor initiation and progression toward metastatic disease, these models are indispensable for preclinical research. GEMMs have successfully been used to validate candidate cancer genes and drug targets, assess therapy efficacy, dissect the impact of the tumor microenvironment, and evaluate mechanisms of drug resistance



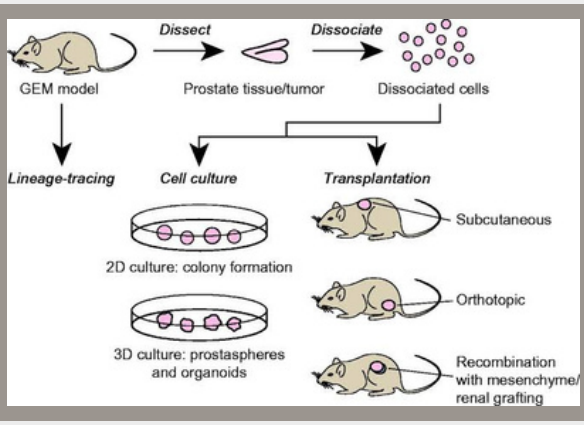
EMBRYONIC STEM CELL-BASED MOUSE CANCER MODELS:



To speed up the generation of novel GEMMs of human cancer, embryonic stem cells (ESCs) can be genetically altered and used to produce cohorts of non-germline GEMMs. An alternative approach is the recently developed GEMM-ESC strategy, which employs ESCs that are derived from existing (multi-allelic) GEMMs.

These GEMM-derived ESCs can be used for rapid introduction of additional genetic modifications and subsequent production of chimeric mice that show the same characteristics as the established GEMM but now contain the additional genetic modification. For example, the GEMM-ESC strategy was used to introduce the Met proto-oncogene in a GEMM of BRCA1-associated breast cancer, which yielded a novel mouse model of BRCA1-deficient metaplastic breast cancer.

Whereas BRCA1-deficient mouse mammary carcinomas showed high sensitivity to the clinical PARP inhibitor olaparib, BRCA1-deficient metaplastic mammary tumors showed intrinsic resistance.



-SANDESH JADHAV

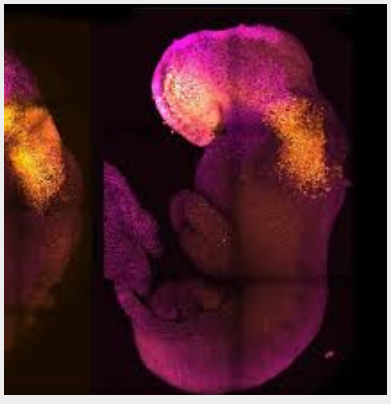
RECENT RESEARCH IN MOUSE MODEL

NEW

● 26TH NOV, 2022 ● MOUSE MODEL

New advances in Stem Cell derived mouse embryo model !

Just in last two month ie. Sep 2022 after announcing the development of a mouse embryo model, complete with beating hearts and the foundations for a brain and other organs, from mouse stem cells, researchers in the laboratory of Magdalena Zernicka-Goetz, Bren Professor of Biology and Biological Engineering, have published new findings about another mouse embryo model reaching similar developmental stages, but created out of only mouse embryonic stem cells. This modification has simplified the protocol and makes the embryo model easier to be adopted in other laboratories.



"CRISPR-SONIC: targeted somatic oncogene knock-in enables rapid in vivo cancer modeling"

Recently group of California Scientist's found this method, that facilitates flexible oncogene knock-in to rapidly model cancer in vivo. Further, while we show CRISPR-SONIC delivery via hydrodynamic delivery in the liver, CRISPR-SONIC cancer modeling may also potentially be applied to other tissues with delivery by lentivirus or adeno-associated virus respectively. Finally, CRISPR-SONIC may also be applied to study other genetic diseases including loss-of-function diseases, by knocking-in of a rescue gene at a safe harbor genomic locus.

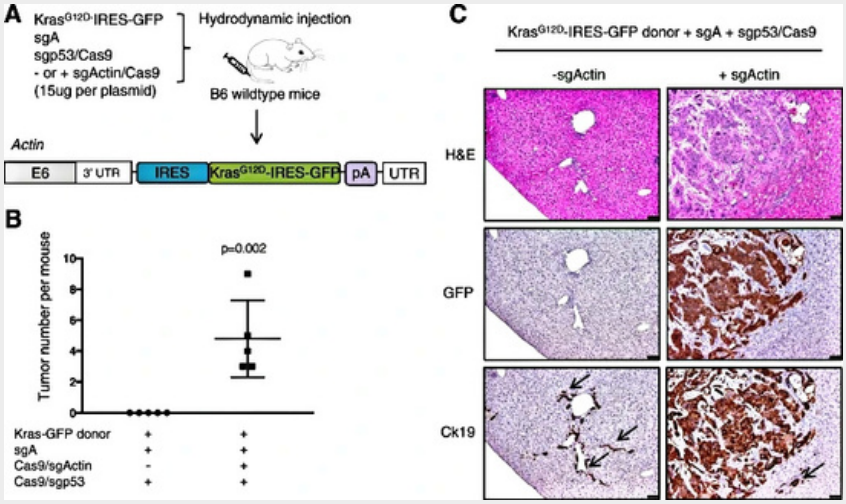


FIG: CRISPR-SONIC enables combinatorial Kras knockin and p53 knockout in B6 wildtype mice. A) Schematic showing the target genomic locus, guide RNAs, and donor plasmid for in vivo integration. B) Quantification of liver tumor per mouse. C) Representative H&E and IHC staining detected GFP-positive and Ck19-positive tumor cells in +sgActin mice.

Herein we reported a flexible system for targeted somatic oncogene knock-in to facilitate in vivo cancer modeling. This method enables rapid knock-in of gain-of-function oncogenes and reporter genes in mice. Next, we expanded our CRISPR-SONIC system to include knock-in of a bioluminescent marker to enable researchers to follow tumor initiation and maintenance in real time. Integrating this luciferase donor allowed us to dynamically quantify tumor size of the model.

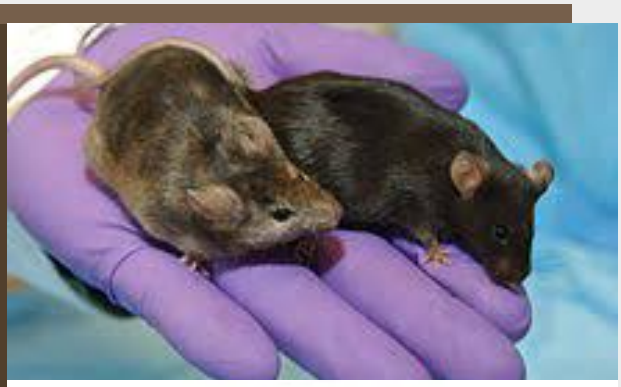
● Www.https://genomemedicine.biomedcentral.com ●

-SANDESH JADHAV

LIMITATIONS

BEHAVIOURAL STUDIES:

LAB ENVIRONMENT VS NATURAL ENVIRONMENT
ANIMALS LACK SELF-CONSCIOUSNESS, SELF-REFLECTION AND CONSIDERATION
HALLMARK OF BEHAVIOURAL DISORDER SUCH AS DEPRESSED MOOD, LOW SELF ESTEEM, SUICIDAL TENDENCY ARE HARDLY ACCESSIBLE IN MICE
HENCE BEHAVIORS LIKE DEPRESSION, AUTISM, SUICIDAL TENDENCY CANNOT BE STUDIED IN MOUSE MOELS.



KNOCKOUT MOUSE

ABOUT 15% OF KNOCKOUTS ARE DEVELOPMENTALLY LETHAL.THIS LIMITS STUDIES TO ONLY EMBRYONIC DEVELOPMENT.
IT IS DIFFICULT TO DETERMINE A GENE'S FUNCTION IN RELATION TO HUMAN DISEASES.
#CUSTOM KNOCKOUT MICE IS VERY EXPENSIVE ($3000-$30000)

NEURO DEGENERATIVE DISORDERS

#MICE LIVE ONLY ABOUT TWO YEARS, WHILE PEOPLE CAN LIVE FOR 80 YEARS OR MORE
NEURO DEGENERATIVE DISORDERS ARE NORMALLY LATE ONSET DISORDERS
#HENCE NOT A GOOD MODEL TO STUDY PARKINSON'S AND ALZHEIMER'S DISEASE AND OTHER DISEASES OF AGING.
#INCAPABLE OF EXPRESSING SOME COGNITIVE HUMANS DISEASE SYMPTOMS

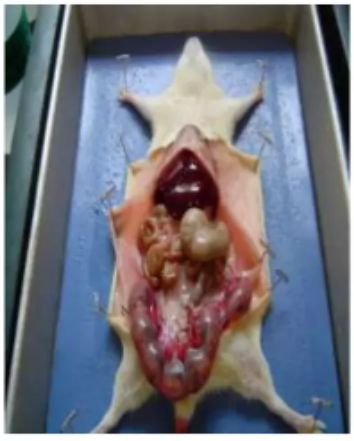
TRANSGENIC MOUSE

TRANSGENE INTEGRATION IS APPARENTLY RANDOM;
MANY EXPERIMENTS REVELS THAT THE GENETIC SURROUNDING OF THE INSERTED TRANSGENIC CONSTRUCT IS MODULATING THE EXPRESSION PATTERN OF THE TRANSGENE ITSELF BOTH QUALITATIVELY AND QUANTITATIVELY.
TRANSGENIC RESCUE OF KNOCKOUT MICE IS TIME CONSUMING, EXPENSIVE AND LABOUR INTENSIVE.

Embryonic manipulation difficult inside the mother
Extra-uterine embryo culture is difficult.
Need to cut open the uterus - ethical issue

FEW MORE LIMITATIONS

EARLY ACTING MUTANT PHENOTYPE DIFFICULT TO STUDY
POOR MODELS TO STUDY INFLAMMATIONS IN HUMANS, A CONDITION PRESENT IN MANY HUMANS.
RESEARCH MICE ARE INBRED AND DO NOT CAPTURE THE GENETIC VARIATION EXISTING IN HUMAN POPULATION
THE RESEARCH WORKS HAVE FOUND THAT THE RESPONSES IN MICE CORRELATED POORLY NOT WITH THOSE IN HUMANS BUT ALSO WITH ONE ANOTHER.
DRUGS THAT HAVE SHOWN PROMISE IN MICE HAVE DONE POORLY IN HUMANS.
FORWARD GENETICS: TO IDENTIFY NOVEL GEES INVOLVED IN EMBRYOGENESIS IS DIFFICULT AS IT IS PROHIBITIVELY EXPENSIVE.



ROHAN

controversy

Mouse Models of In... Worthless. Now We Know.

by Piper Hoffman | February 17, 2013 | 2:30 pm

Posted by Derek

Surprising result: Mice are not good models for (some!) ilnesses in humans.

Neuroprotection through Delivery of Glial Cell Line-Derived Neurotrophic Factor by Neural Stem Cells in a Mouse Model of Parkinson's Disease

Trichostatin A increases the levels of plasma gelsolin and amyloid beta-protein in a transgenic mouse model of Alzheimer's disease

β-Amyloid peptides enhance α-synuclein accumulation and neuronal deficits in a transgenic mouse model linking Alzheimer's disease and Parkinson's disease

MICE TRIALS DID NOT TRANSLATED TO HUMAN!

POOR MODEL TO STUDY INFLAMATION HUMANS, A CONDITION PRESENT IN MANY HUMANS

MICE ARE RESISTANT TO INFECTIONS AND INFLAMATIONS.

MICE HAVE LIVED FOR MILLENNIA IN ENVIRONMENTS TEAMING WITH MICROBES AND THEY SHORT GESTATION PERIODS AND LARGE LITTERS. THEY SHOWS THEY HAVE EVOLVED WITH DIFFERENT STRAGTERGIES FOR DEALING WITH INFECTIONS.

RESEARCH MICE ARE INBRED AND DO NOT CAPTURE THE GENETIC VARIATION EXISTING IN HUMAN POPULATION!!!

THE RESEARCH WORKS HAVE FOUND THAT THE RESPONSES IN MICE CORELATED POORLY NOT WITH THOSE IN HUMANS BUT ALSO WITH ONE ANOTHER

BIOMEDICAL RESEARCH

Inflammation debate reignites

Reanalyzing same data, paper challenges controversial study casting doubt on use of mouse models

ROHAN