Knockout mice and their use in science research

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Knockout Mice

They are not mice that won a boxing match. Instead, knockout refers to genetically altered mice with an inactivated gene. To study the effects and mechanisms of a gene, looking at family members or the protein sequence produced by the gene is not sufficient. Even close relatives have a large number of variations in genes, while protein function is hard to determine from the sequence alone.[1] As such, scientists must isolate the gene; however, it is unethical to purposely infect a human with a disease. Thus, knockout mice are used because their genome is 99% similar to humans' genome, have short life spans, and high reproductive capabilities.[1]

Knockout mice are usually used for recessive diseases.[1] Humans and mice inherit two copies of a gene at a specific location or locus. These copies are called alleles. To produce a recessive disease, both copies must be the same recessive allele (compared to dominant alleles that only need one copy to produce its effects). The scientific term for having two identical alleles is homozygous. For dominant diseases, researchers can insert the dominant allele without changing the rest of the genome, but this would not work for recessive diseases.[1] As such, knockout mice require researchers to "knock out" or inactivate both copies of their gene.

Production

To produce knockout mice, murine embryonic stem cells (ES cells) are harvested from mice embryos and would be genetically altered using either a modified viral vector or bacterial DNA.[1,2] This can be done through two different methods.

Homologous recombination

This utilizes the DNA repair mechanism in the body that replaces damaged sequences with similar DNA.[1] Vectors would have a long strand of DNA that is homologous or the same as the original sequence, except for the inactive or altered portion of the gene. This sequence would be exchanged with the original;[2,3] however, there is a low success rate. To make sure the replacement worked, researchers often include markers or reporter genes in the sequence that signals if the DNA was exchanged in the right place.[2]

Gene trapping

This does not directly target a certain gene. Instead, the altered DNA would be inserted randomly, and this insertion would prevent the RNA splicing and expression of that gene, effectively knocking it out.[3] This altered DNA would often contain a reporter gene that would tell the researchers where the sequence was inserted.[3] The benefit to this method is that the specific DNA sequence of the gene does not have to be known, but it can potentially knock out a large variety of genes so extensive testing is needed to determine which where the DNA sequence is affected.[3]

Once the sequence is inserted into the ES cells, it would be injected into blastocysts in the uterus of a female mouse to produce a chimeric mouse.[1,2] These ES cells can develop into a variety of cells, including

germ cells, which contains the DNA passed on to its offspring. However, at this point, the chimeric mouse still has some normal tissue, so more crossbreeding is needed to create a homozygous (both copies of the gene are the same) knockout mice.[2]

Applications

Knockout mice are incredibly useful as models of diseases, including cancer, diabetes, and Parkinson's, where researchers can see how the gene functions or test pharmaceuticals and treatments on the mice. They are especially helpful for developmental genes, such as SF-1 that is essential for adrenal gland and gonad development, or single gene mutation diseases, such as cystic fibrosis.[2] These mice are often named for the gene they knock out. For example, the Cx3cr1 knockout mice (also symbolized by $Cx3cr1^{-/-}$) affect the microglial chemokine receptor, so they were used to study microglia in neuron loss in Alzheimer's disease.[4]

Bottom line: Knockout mice are useful for testing the effects of a specific gene.

References

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