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Models for Translational Research of Human Diseases

ABSTRACT

Human disease models, including cell models, are important platforms in translational research studies to understand the pathophysiology of human diseases. The subject is vast as many models and diseases are involved. This essay will focus on the following models i.e. *Saccharomyces cerevisiae* (baker's yeast), *Schizosaccharomyces pombe* (fission yeast), *Caenorhabditis elegans* (roundworm), *Drosophila melanogaster* (fruit fly), *Danio rerio* (zebra fish) and *Mus musculus* (laboratory mouse), which are being used in such human diseases such as cancer, metabolic diseases, inflammation, infection, and neurodegenerative conditions. With the impact of CRISPR and next-generation sequencing technologies, disease modelling is stepping up the understanding of molecular mechanisms of human diseases. This in turn is helping in the theranostic management (diagnosis, prognosis and therapy) of the latter and paving the way to more personalized precision medicine.

INTRODUCTION

A disease model is an organism or its cells that display a pathological process that is observed in the human disease. Examples of well-known model organisms that will be discussed in this essay are *Saccharomyces cerevisiae* (baker's yeast), *Schizosaccharomyces pombe* (fission yeast), *Caenorhabditis elegans* (roundworm), *Drosophila melanogaster* (fruit fly), *Danio rerio* (zebra fish) and the laboratory mouse (*Mus musculus*). There are many others of course.

Indeed, human disease models are many and diverse and their utility depends on the investigations which need to be carried out. The choice of the model exploited is generally a trade-off based on the degree that the model mimics the disease and how easy the model platform can be manipulated. Normally, these organisms have certain common characteristics and Table 1 lists some of them.

Table 1. Some common characteristics of disease models.

Studied extensively
Relatively easy and cheap to maintain and breed in large numbers under laboratory conditions
Relatively short generation time (interval from birth to reproduction capability)
DNA can be genetically mutated, thus allowing studies of a specific characteristic or disease
Similar genes to humans (homology)

Homology refers to the similarity between genes in different taxa. A gene is called homologous because it has been inherited in species that have evolved from a common ancestor. Homologous genes code for homologous proteins that have similar functions. When it comes to human disease models, the models are used because they have human homologous genes. And the more this homology is, the more chance that the research could (but not always) be translated to humans.

Human disease models are becoming increasingly important to understand the fundamentals of biology and medicine and are paving the way to better understand genetic susceptibility to disease. Methods that target and edit genes are enabling researchers to create mutant alleles of the genes under scrutiny and so help in studying gene function. Unprecedented advances in genomics and gene sequencing (like next-generation sequencing technology) are also helping in the translation of research findings using model organisms. Other platforms that are helping researchers to interrogate more precisely gene function include CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) gene-editing technology, knockout methods, and the availability of tools to mine data from several data banks (genomic, transcriptomic, proteomic, metabolomic) from various organisms including those of humans. This integration allows more precise translation from models to humans.

Translation is not always possible due to various reasons. One reason is that biological systems exhibit complexity and redundancy. Another two reasons are that human disease has its own pathophysiological heterogeneity and each disease encompasses subpopulations of patients with somewhat different pathophysiological mechanisms. Also, many candidate molecules in clinical trials fail due to pharmacokinetic challenges relating to absorption and distribution in the target organ.

Yeast (*Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*)

Yeasts are simple eukaryotes. The main yeasts that are valuable, versatile and powerful disease model organisms are *Saccharomyces cerevisiae* (baker's yeast) and *Schizosaccharomyces pombe* (fission yeast). Their genetic tractability, short generation time and easy and cheap genetic manipulations are pivotal in the large worldwide network of research to understand gene variants of different human diseases. *Saccharomyces cerevisiae* was the first eukaryote to have its genome fully sequenced and functionally annotated. This led it to serve as a leading reference genome when annotating new genes and when studying other complex organisms.

Melanie Lee and Paul Nurse (1987)[1] published a seminal paper on *Schizosaccharomyces* revealing work on a key regulator of the cell cycle, specifically CDK (cyclin-dependent kinase). This earned Nurse a Nobel Prize in Physiology or Medicine in 2001, which was shared with Tim Hunt and Lee Hartwell. Elizabeth Blackburn, Carol Greider and Jack Szostak also used yeast and were awarded a Nobel Prize in Physiology or Medicine in 2009 for their work on telomere and telomerase. These molecular underpinnings using yeast later had clinical repercussions in cancer as cyclin-dependent kinases (and their partners, the cyclins) are molecular targets for treatment.

These unicellular eukaryotes are also important models to study aging and neurodegenerative disease like Parkinson's, Alzheimer's, Huntington's and motor neuron diseases. The pathological processes that underlie these conditions, namely impaired autophagic protein degradation, impaired mitochondrial function, deviant programmed cell death mechanisms, and misfolding and aggregation of culprit proteins, are all found in yeast.

Research using yeast is still ongoing. Indeed, the importance and function of the non-protein coding elements (once called the 'dark matter of the genome') of this eukaryotic genome are being found to be also essential to the fitness of the organism. Grech et al.[2] using saturating transposon mutagenesis, interrogated the *Schizosaccharomyces pombe* genome. They found that the transposon insertions had fitness effects in 80% of the noncoding regions. Specifically, they also found 85 necessary non-coding RNAs during the vegetative growth of the yeast and 218 during its ageing phase. Research similar to this has propelled more studies to underpin the functions of ncRNAs, and some discoveries have already been translated into the clinical setting as biomarkers and non-coding RNA-based therapy.[3]

Roundworm (*Caenorhabditis elegans*)

Caenorhabditis elegans is a nematode worm and it also has been used to study various human diseases. Table 2 shows the names of the researchers who won a Nobel Prize stemming from research using this worm. This goes to show the importance of this worm in research.

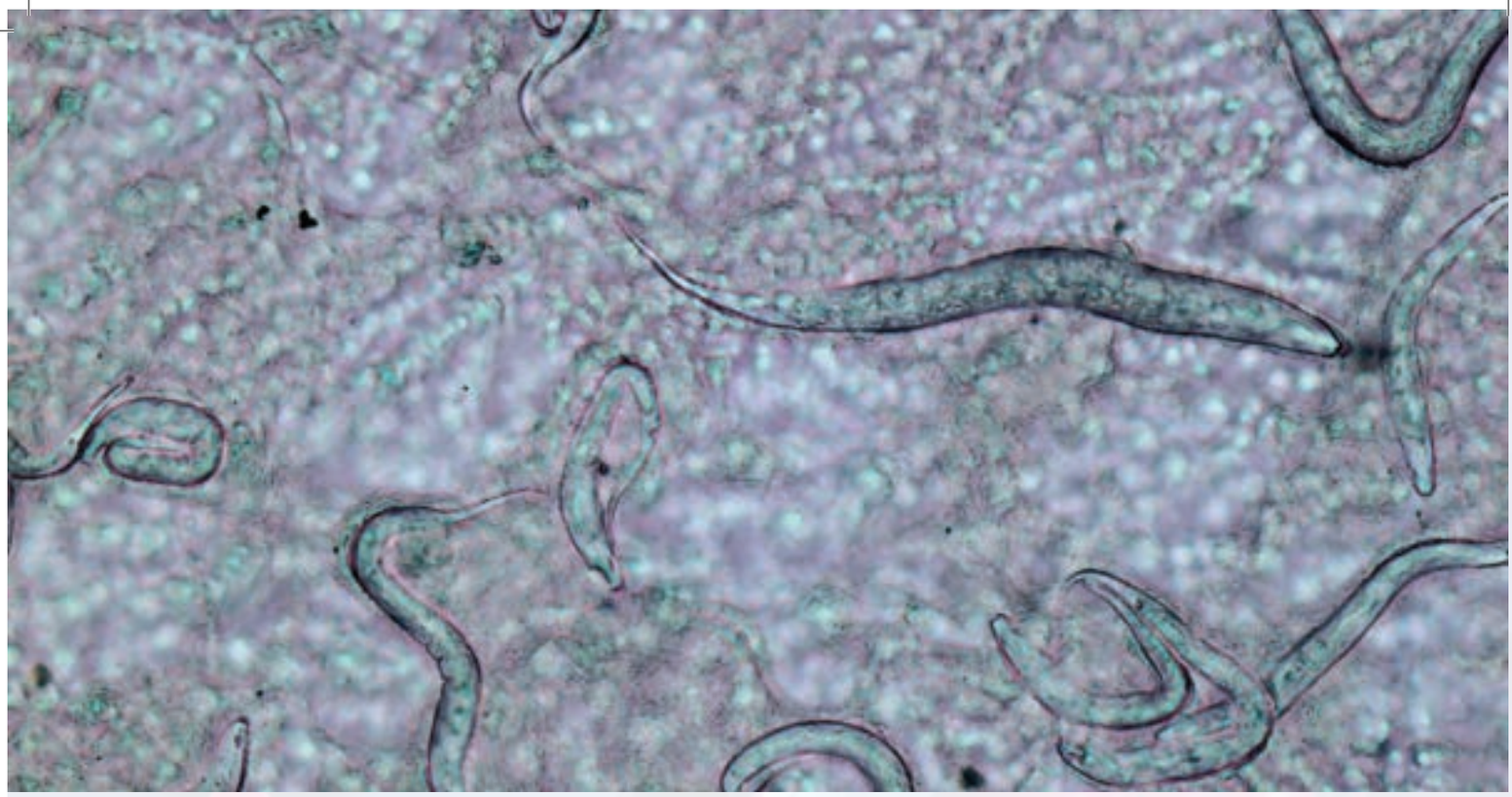
Table 2. Nobel Prize laureates and their discovery.

Year, Nobel Prize Winners	Discovery	Paper
2002, Sydney Brenner, Robert Horvitz and John Sulston	Developmental biology and cell death machinery	4,5,6
2006, Andrew Fire and Craig Mello	RNA interference (RNAi)	7
2008, Osamu Shimomura, Martin Chalfie and Roger Tsien	Green Fluorescence Protein (GFP)	8

Table 3 shows some of the diseases that this worm was used as a model.

Table 3. Selected studies using Caenorhabditis elegans.

Human Disease or condition	Study	Reference
Autism	Neuroligin dependence of social behaviour	9
Retinitis pigmentosa (RP)	Drug screening and identification of modifiers	10
Alzheimer's disease	Neuroprotective effect of human Vps41 protein	11
Parkinson's disease	Modulation of cholesterol metabolism in neurons	12
Oncology	Anti-cancer potential of etodolac, itraconazole, disulfiram and ouabain	13



Autism is characterised by disrupted social behaviour. Rawsthorne et al.[9] used *C. elegans* to study a gene that codes for neuroligin, which is associated with autism. Neurologin is a protein in the postsynaptic membrane involved in the connection of neurons at synapses. They found that neuroligin is specifically important in the recognition and processing of social signals.

Kukhtar et al.[10] used CRISPR/Cas technology to create mutant strains of *C. elegans* to target splicing-related genes associated with retinitis pigmentosa (RP). They tested a set of RNAi clones on these genes and found that their partial inactivation might alter the course of the disease. Also, they found that dequalinium chloride (an active ingredient in various medications) could be harmful to a type of RP.

In their study, Griffin et al.[11] compared two transgenic models of *C. elegans* to show the neuro-protective effect of human Vps41 (vacuolar protein sorting 41), which is a protein involved in the trafficking of lysosomes. Their work aimed to find targets that can halt the degeneration of neurons in Alzheimer's disease. On the other hand, through their findings using *C. elegans* as a model, Zhang et al.[12] showed that modulating cholesterol metabolism in neurons can reduce the neurotoxicity of alpha-synuclein. The latter, which are peptised, are the major components of Lewy bodies. They even propose this regulation as a prospective treatment for Parkinson's disease.

Medina et al.[13] used mutant strains of *C. elegans* and showed the anti-cancer potential of 4 drugs, specifically etodolac, itraconazole, disulfiram, and ouabain. Their investigation could lead to a drug repurposing strategy.

The immune system has two pillars, (i) innate immunity and (ii) adaptive immunity. Given that *C. elegans* has only the innate immune response, it has been used to study this response to pathogens. The standard feed for *C. elegans* is *E. coli*. The latter can be swapped with a pathogen like *Salmonella enterica*. Investigations of this setup showed an immune response of the host involving the p38 MAPK (mitogen-activated protein kinase) signalling cascade.[14] This pathway generates various biological effects in response to diverse external signals. The study also showed that the crucial factors for the virulence of *Salmonella Typhimurium* are an expression of PhoP/PhoQ and SPI-1 (*Salmonella* pathogenicity island 1).

In keeping with the fact that the ageing process is a common factor for many diseases, *C. elegans* has been exploited to study ageing, especially the use of anti-ageing drugs for a healthier ageing phenotype and lifespan modulation. Ziehm et al.[15] discuss a pipeline for candidate compounds including some repurposed drugs (like imatinib), with which further investigations could be used for healthier ageing in humans. Wang et al.[16] found anti-oxidant and anti-ageing effects in extracts from hawthorn berries. The hawthorn tree's berries and leaves have long been used in traditional medicine for their various health benefits. Wang et al. found that the lifespan of *C. elegans* was increased by 28% when treated with the extract and also improved resistance to stress caused by ultraviolet radiation and heat. They even proposed that these health benefits were mediated by the insulin/insulin-like growth factor-1 (IIS) signalling pathway. The latter connects nutritional status to important functions like growth, metabolism, reproduction and ageing. Such investigations are still at an early stage but they are being honed into better pipelines which will ultimately be translational for the benefit of humans.

Fruit fly (*Drosophila melanogaster*)

Table 4. List of Drosophilists who won a Nobel Prize.

Drosophilists who won a Nobel Prize	Discovery
Thomas Hunt Morgan	Chromosomes contain linear arrangements of genes
Hermann Joseph Muller	X-rays can cause mutations
Christiane Nusslein-Volhard Eric Wieschaus Edward B. Lewis	Genetic control of early development of the embryo
Jules A. Hoffmann	Activation of the Innate immunity
Michael Rosbash Jeffrey C. Hall Michael W. Young	Molecular mechanisms of circadian rhythm

Table 4 lists drosophilists who won a Nobel Prize. Again, this shows the importance of *Drosophila melanogaster*. Indeed, it is another extensively studied and tractable genetic model organism which underpins the molecular pathways of a wide range of human diseases. Importantly, about 75% of genes that cause disease in humans have a functional homolog in this fly.

Drosophila has been used to study epigenetics associated with ageing and cancer. It is also being exploited in nutrition research to study the molecular mechanisms of diabetes and obesity. Moreover, Drosophila is used in research associated with COVID-19. It has also been exploited for drug discovery and repurposing. For example, Alli et al. [17] found a potential anti-lithogenic compound, arbutin, for kidney stones. Bangi et al.[18] fished a two-drug cocktail of trametinib plus zoledronate as a personalized treatment for a patient with metastatic colorectal cancer showing a KRAS (Kirsten rat sarcoma)-mutant phenotype. KRAS-mutant phenotype is associated with marked aggressiveness and poor prognosis. Bangi et al. analysed the cancer's genome and fished 9 perturbed culprit genes. A transgenic model of the fly was developed containing the mutated orthologs of the 9 perpetrator genes. Subsequently, a robotic-based screening was employed and this led to the identification of trametinib plus zoledronate as a potential treatment. The patient showed an acceptable significant response. In their paper they propose that such a personalized approach can be a way forward for refractory cancer, offering hope for patients with an otherwise poor prognosis.

Zebrafish (*Danio rerio*)

Another relevant model which has gained prominence in biomedical research of human disease is the zebrafish model. This is because its biology permits research of all developmental stages. Indeed, the internal organs can be visualised non-invasively as its embryos and larvae are optically transparent and allow real-time imaging of normal and pathological development. In fact, every cell can be seen and studied. Another advantage is that one can easily create an efficient and cost-effective system because zebrafish are relatively easy to maintain in the laboratory in large numbers. Importantly, like the other disease models mentioned so far, there is a high conservation of genes between the human genome and that of the zebrafish. Specifically, 70% of human genes have a counterpart in the zebrafish. And considering human disease genes, 84% have a zebrafish counterpart.

Specifically, the zebrafish is being exploited as a disease model to understand the mechanisms of COVID-19. Angiotensin-converting-enzyme-2 (ACE2) is an important protein in the renin-angiotensin-aldosterone system (RAAS) pathway. ACE2 is the receptor SARS-CoV-2 targets to enter and infect human cells. Kim et al.[19] studied the effect of RAAS inhibitors (aliskiren, olmesartan and captopril) on ACE2 expression in various organs of the zebrafish. They found that ACE2 expression is organ-specific and thus the benefit-risk analysis of using RAAS inhibitors in COVID-19 should be thoroughly investigated. Costa et al[20] also used zebrafish to study anosmia in Covid-19 and confirmed that the virus attacks the olfactory cells.

Laboratory Mouse (*Mus musculus*)

The disease models mentioned so far have their limitations because of differences in physiology and anatomy when compared to humans. This warrants the use of mammalian models for preclinical testing. Testing in mammalian models like the mouse also ascertains the pharmacodynamics and the pharmacokinetics of potential drugs that have been fished out using the other models. Still, it must be said that the experimental response in mice can differ from that in humans. For example, many cancer treatments that cure mice often have inadequate effect in humans and many prospective candidates never make it to the market. Nonetheless, the mouse is a relatively reliable model for humans and its genome is about 85% identical to the human genome.

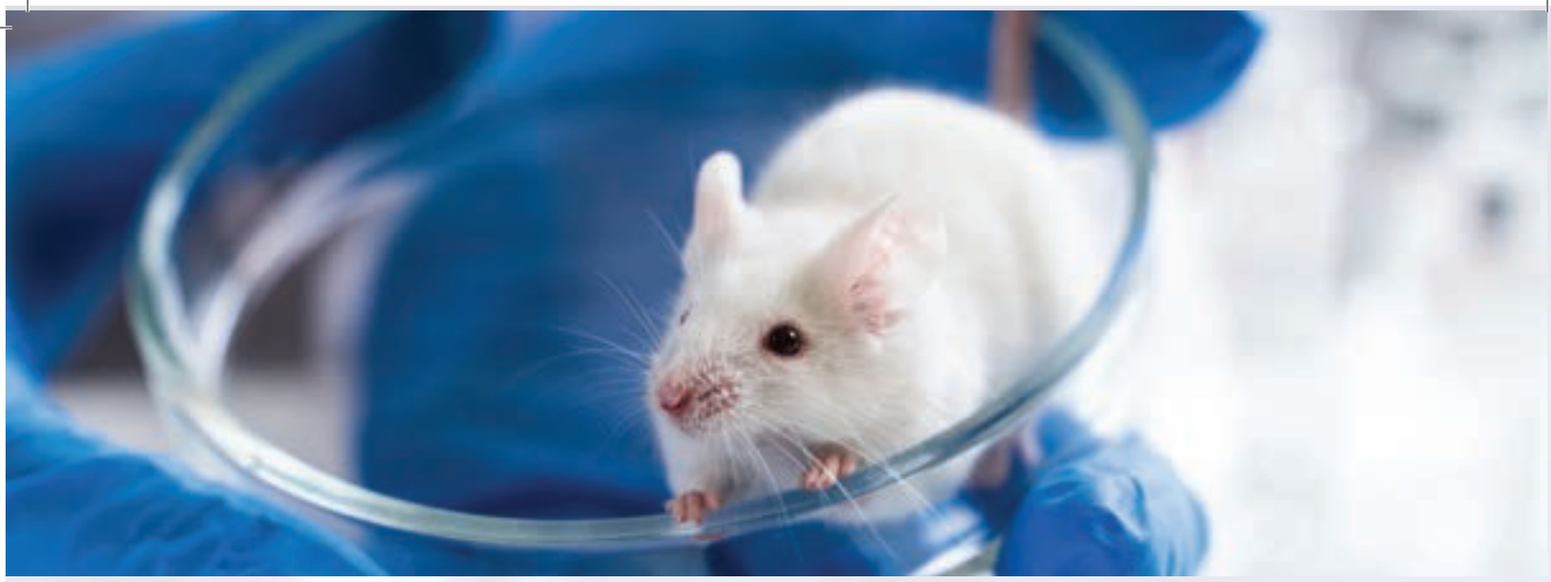


Table 5. Nobel Prize laureates and their contribution.

Year	Nobel Laureate	Contribution
2018	Tasaku Honjo James P. Allison	Cancer therapy (inhibition of negative immune regulation)
2016	Yoshinori Ohsumi	Autophagy mechanisms
2012	John B. Gurdon Shinya Yamanaka	Reprogramming mature cells to become pluripotent
2008	Harald zur Hausen	Human papilloma causing cancer of the cervix
1997	Stanley B. Prusiner	Prions discovery
1988	Gertrude B. Elion James W. Black	Principles for drug treatment

Breakthroughs using the laboratory mouse have netted about 17 Nobel Prizes. Table 5 shows the year and some of the names of the noble laureates who won a Nobel Prize. Again, this is a showcase of the biomedical research that has been vastly carried out using mice.

When it comes to behavioural studies, the laboratory mouse (*Mus musculus*) is one of the preferred models as it shares similar behavioural aspects with humans including sexual behaviour, anxiety, hunger, aggression, memory and circadian rhythm. There is also resemblance in anatomy, biochemistry, and molecular mechanisms. Over the years several mouse models have been created. Grayson et al.[21] used two such mouse models specifically to study schizophrenia, i) the 'prenatal restraint stress model' and ii) the 'chronic methionine mouse model'. They analysed the DNA methylation profiles in the adult offspring and found behavioural, epigenetic and other biochemical deficits. Such studies are providing solid evidence that early-life adversity and other environmental factors can mediate long-lasting epigenetic modifications in the brain, which are conducive to mental ill-health.

Using another mouse model Dr Yuan Shi and Mochen Cui et al.[22] found the underlying mechanism for the cognitive impairment and dementia associated with the long-term use of benzodiazepines. They found that there is loss of neuron connections. Specifically, they found loss of neuronal dendritic spines and synapse degradation, resulting from activation of microglia (immune cells of the central nervous system) which occurs when diazepam binds to TSPO (translocator protein), a protein on microglial organelles.

Winkler et al.[23] created a mouse model to study COVID-19 infection. As already pointed out, SARS-CoV-2 needs to bind to ACE2 receptors to infect the human cells. However, murine ACE2 receptor is structurally different from the human ACE2 and so Sars-CoV-2 cannot enter murine cells. Winkler et al. created a mouse that expresses human ACE2 in its epithelial cells. They then used this ACE2-transgenic mouse to model infection by SARS-CoV-2. They introduced the virus intranasally and found that the virus infected the lungs and then spread to other organs. Four days after infection, the lungs were compromised due to an infiltration with monocytes, neutrophils and activated T cells. They proposed that their mouse model could be used to further study COVID-19 infection and anti-viral treatments.

Further research by Tasuku Honjo and James Allison focused on apoptosis. They found that apoptotic T cells in mice that lacked an important receptor molecule, called 'programmed death molecule-1' (PD-1), go on to develop autoimmune diseases like type 1 diabetes, glomerulonephritis and myocarditis. PD-1 is an immune checkpoint protein and its upregulation leads to enhanced immuno-suppression. This research has paved the road to a promising new avenue for cancer immunotherapy through the use of checkpoint inhibitor drugs. Indeed, these drugs are offering hope in patients with advanced cancer. The cancers vary from advanced cutaneous squamous-cell carcinoma[24] to non-small-cell lung cancer.[25]

Cellular Models

A cell disease model can be a collection of cells derived from a tissue with a known disease. These cells are then cultured and researched *in vitro* to gain insights of the pathophysiological mechanisms specific to the disease. Such models are also being exploited to help discover molecular targets for treatment. They can also be used for testing out new treatments and thus help in developing new safer therapies.

Another type of cell disease model involves the use of induced pluripotent stem cells (iPS cells). For example, such cellular reprogramming platforms are offering insight in understanding neuropsychiatric disorders. In their review, Seshadri et al. [26] hypothesise that induced pluripotent stem cells (iPSCs) and their neural derivatives may be used to understand schizophrenia. Viswanath et al.[27] similarly reviewed cellular models to study bipolar disorders.

One important advantage of the traditional iPSC technology, when used to model human diseases, is that the induced cells have the whole genome of the donor and this makes it fit to dissect diseases caused by genetic errors. This platform is becoming more valuable when combined with CRISPR/Cas9 gene editing and genome-wide association studies. However, when it comes to studying epigenetics it faces a problem, namely that during the process of reprogramming, the epigenetic memory is erased (epigenetic erasure).[28] Thus mental illnesses that are epigenetically modified by environmental factors need to be studied by a sister technology called 'transdifferentiation' to generate functional-induced neurons (iNs).[29] Transdifferentiated cells seem to maintain the original epigenetic landscape.[30,31] However, such studies are still in their infancy.

CONCLUSION

The development of various translational disease models has been and still is vital to the comprehensive and expanding investigations into the diverse pathogenic mechanisms of disease and also, in bettering the identification of theranostic targets. The choice of the model employed is often a trade-off based on how much the disease model reflects and translates the human disease and on how easily the model platform can be manipulated. Surely, the integration of various advancing fields (like genomics, transcriptomics, proteomics, metabolomics and bioinformatics), together with automation technologies, when applied to such model organism systems, will enhance the discovery of biomarkers and of new and safer drugs.

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