DRUG DISCOVERY

Therapeutic Strategies in Animal Models

The Case of Alzheimer's Disease

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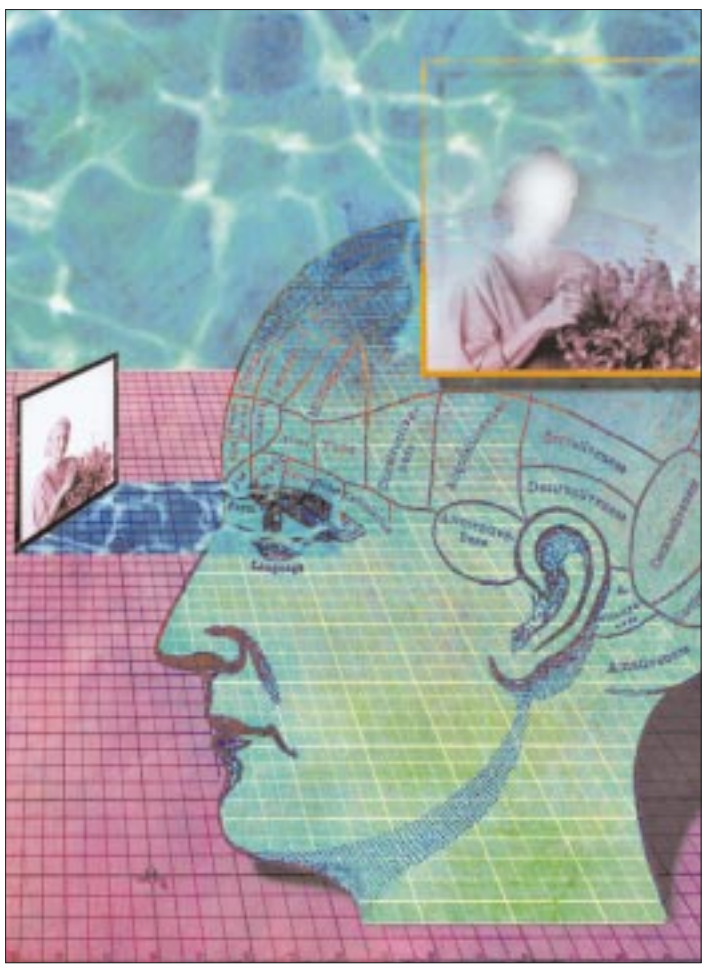
In recent years, many experts in the pharmaceutical industry, the FDA, and the National Institutes of Health (NIH) have identified the need for improved animal models as a critical bottleneck in drug discovery and development.

The FDA white paper *Innovation or Stagnation: Challenge and Opportunity on the Critical Path to New Medical Products* (March 2004, www.fda.gov/oc/initiatives/criticalpath/whitepaper.html) cites the poorly understood clinical relevance and limited predictive value of many animal models, which hinder effective drug discovery and development.

In our report, *Model Animal Systems: Emerging Applications and Commercial Opportunities in Drug Discovery and Development* (Cambridge Healthtech Advisors, June 2004), we review recent progress in the application of model organisms to drug discovery and development.



Animal models remain an important component in the development of therapeutic strategies for neurodegenerative diseases, such as Alzheimer's disease.

This includes case studies on the development and use of disease-specific animal models to develop novel therapeutic strategies. These case studies include applications of animal models to diseases of aging, neurodegenerative diseases, cancer, and cardiovascular diseases.

Animal models have been and continue to be important in developing therapeutic strategies for neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD). Much about the pathogenesis of these diseases remains unknown. However, a common feature of disease pathways in these diseases is the role of abnormal, toxic proteins *(Table)*. These proteins aggregate and are deposited in extracellular plaques or intracellular inclusion bodies, which are characteristic of each of these diseases.

Researchers' knowledge of these disease pathways has been gained by a combination of pathology studies in postmortem human tissues, human genetics, and studies in animal models. In AD, patients typically exhibit two types of brain lesions: extracellular "senile plaques" made up largely of b-amyloid (Ab) peptide, and intracellular "neurofibrilary tangles," made up largely of the cytoskeletal protein tau.

By studying rare, familial cases of early-onset AD, human geneticists have identified three disease genes in these conditions—genes for amyloid precursor protein (APP), and for two presenilins, PS1 and PS2.

The presenilins are transmembrane proteins that are involved in the cleavage of APP to produce Ab. With wild-type presenilins and wild-type APP, APP is cleaved so as to give predominantly the 40-amino-acid form of Ab, with a small amount of a 42-amino-acid form.

However, mutant forms of any of the three genes results in formation of greater amounts of the 42-amino-acid form of Ab (Ab42), which more readily aggregates and forms amyloid plaques.

Amyloid Hypothesis

Because of these findings, most AD researchers have focused on the APP processing pathway or on aggregation of Ab as intervention points for therapeutic strategies. The hypothesis that this is the central AD disease pathway is called the "amyloid hypothesis."

Nevertheless, although specific mutations in amyloid pathway genes appear to explain the formation of senile plaques in early-onset familial AD, it is not known how dysfunction in the amyloid pathway occurs in the more common, late-onset sporadic form of AD.

Several researchers have constructed transgenic mouse strains that express mutant forms of human Ab. For example, researchers at **Elan** (Dublin) developed a transgenic mouse model, called the PDAPP mouse, which overexpresses the V717F (valine at residue 717 substituted by phenylalanine) mutant form of human APP.

This mouse model develops amyloid plaques resembling those in human AD in an age-dependent and brain-region dependent manner. In 1999, Elan researchers published a study showing that immunization of the mice with Ab42 ameliorates plaque formation and other neuropathological changes.[1] This led to Elan's current collaboration with **Wyeth** (Madison, NJ) to develop immunotherapies for AD.

Elan and its collaborators also determined that the enzymes beta-secretase and gamma-secretase are involved in the APP processing pathway.[2,3] The gamma-secretase study involved using the PDAPP model.

The researchers administered an inhibitor of gamma-secretase orally to the mice, and demonstrated reduction of Ab levels in the brain in a dose-dependent manner. Beta- and gamma-secretase are targets for drug discovery in Alzheimer's disease at Elan and several other companies (e.g., **Bristol-Myers Squibb, GlaxoSmithKline**).

Researchers have also been using the nematode *Caenorhabditis elegans*, the fruit fly *Drosophila melanogaster*, and the zebrafish *Danio rerio* to study disease pathways in AD. Disease models based on these organisms allow researchers to apply powerful genetic methods that are not possible in mammalian systems to the study of disease pathways, and to target identification and validation.

They also allow for high-throughput screening of drugs in vivo. However, targets identified and/or validated in these systems, as well as compounds that modulate them, must be confirmed in mammalian models, which are also necessary for preclinical studies.

Presenilin Homologues

Presenilin homologues are found in *C. elegans, Drosophila,* zebrafish, and the mouse. These proteins function in the Notch pathway. In this pathway, presenilins are involved in proteolytic cleavage of the Notch intracellular domain, and the resulting proteolytic fragment can enter the nucleus and carry out signaling.

The Notch pathway is involved in various developmental processes in *C. elegans, Drosophila*, zebrafish, and in mammals. For example, in adult mammals this pathway is involved in hematopoiesis, immune cell differentiation, and numerous other processes involving self-renewal of cells and tissues from stem or progenitor cells.

In mammals, presenilins are involved in pathways for proteolytic processing of intracellular domains of both Notch and of APP.

Gamma-Secretase

In recent years, researchers at **Exelixis, Schering-Plough Research Institute,** and several universities, using the *C. elegans, Drosophila*, zebrafish, and murine Notch pathway systems, have conducted studies that resulted in the identification of the components of gamma-secretase.

In *Drosophila* and in mammals, gamma secretase is a membrane protein complex that contains three other components in addition to presenilins. Presenilins undergo endoproteolysis to yield an N-terminal and a C-terminal fragment; these associate with the other components of gamma-secretase.

Drosophila or zebrafish presenilins can substitute for their human homologues in transgenic human cells—they undergo proper endoprotolytic processing and yield gamma-secretase activity.[4,5]

Another study showed that treatment of zebrafish with gamma-secretase inhibitors affected embryonic development in similar ways to mutants in Notch pathway genes. This suggests that at least some gamma-secretase inhibitors may inhibit essential Notch pathway-mediated functions.

Such compounds would not be good drug candidates. However, one research group used the zebrafish model to identify small-molecule drugs that inhibit gamma-secretase-mediated production of Ab but not its activity in the Notch pathway.

In contrast, a beta-secretase knockout mouse constructed by researchers at Elan, Pharmacia (now **Pfizer**), and Artemis (now **Exelixis Deutschland**) displayed an apparently healthy phenotype. Brains and cultured cortical cells from these knockout mice produced much less Ab from APP. These studies suggest that beta-secretase may be a better drug target than gamma-secretase.

Although most studies of AD focus on the amyloid hypothesis, some researchers believe that the hypothesis may not fully explain the pathogenesis of the disease, and that animal models based on the amyloid hypothesis may not adequately model AD.[6] The extent to which an animal model models a human disease is a general and important issue.

Lewy Bodies

In addition to the Ab containing "senile plaques" and tau-containing neurofibrilary tangles seen in AD brain, in most cases Lewy bodies containing a-synuclein are also seen. (As shown in the *Table*, Lewy bodies are characteristic of PD.) Also characteristic of AD are cholinergic neuron, synaptic loss, and inflammation.

Mouse models of AD that overexpress mutant human APP develop Ab-containing plaques, and also exhibit tau and a-synuclein positive neurites. However, they do not exhibit neurofibrilliary tangles or Lewy bodies, or neurodegeneration or deficits in cholinergic function.

Some researchers therefore hypothesize that these mice model only the early stages of AD, and that the formation of Ab plaques alone may not cause neurodegeneration without the formation of tau and/or a-synuclein inclusions.

Several research groups have constructed mouse models that exhibit both amyloid plaques and other types of inclusions seen in human AD. One such model is transgenic for both human Ab and human a-synuclein.[7] These mice exhibited severe deficits in

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Aggregation and Deposition of Abnormal Proteins in Alzheimer's and Parkinson's Diseases

Disease	Protein deposits	Toxic protein	Disease genes
Alzheimer's disease	Extracellular plaques	Ab	APP*
		Tau	Presenilin 1†
		a-Synuclein (?)	Presenilin 2†
	Intracellular tangles		
Parkinson's disease	Lewy bodies (cytoplasmic inclusions)	a-Synuclein	a-Synuclein*
			Parkin†#
			UCHL1†#

*Mutations associated with a toxic gain of function; †Mutations associated with a loss of function; # Parkin and UCHL1 are involved in pathways for removal of misfolded proteins; researchers hypothesize that mutations in the genes for these proteins result in accumulation of toxic, misfolded a-synuclein.

Abbreviations:
Ab: Beta amyloid protein; APP: Amyloid precursor protein; UCHL1: Ubiquitin carboxy-terminal hydrolase L1

Source: Haberman Associates

Model Systems

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learning and memory, and age-dependent degeneration of cholinergic neurons.

They also had more a-synuclein-containing neuronal inclusions than mice transgenic for a-synuclein alone. A second mouse model was doubly transgenic for human APP and human mutant tau.[8]

Singly tau transgenic mice developed a progressive motor disturbance. Mice doubly transgenic for APP and mutant tau demonstrated greater numbers of tau-containing neurofibrilary tangles than singly mutant tau-transgenic mice. They also demonstrated neurodegeneration and age-dependent motor disturbance.

Mouse models that more closely resemble the pathology of human AD may enable researchers to better understand the full spectrum of pathogenic pathways in AD. They may also enable them to develop drugs that can treat AD in its various stages.

The above studies illustrate two important strategies that researchers have been applying to various diseases: utilizing invertebrate and zebrafish models that allow the use of powerful genetic and high-throughput screening methods, and developing mammalian models that more closely mimic human disease.

These strategies are expected to enable researchers to gain a greater understanding of disease pathways and develop new approaches to drug discovery. GEN

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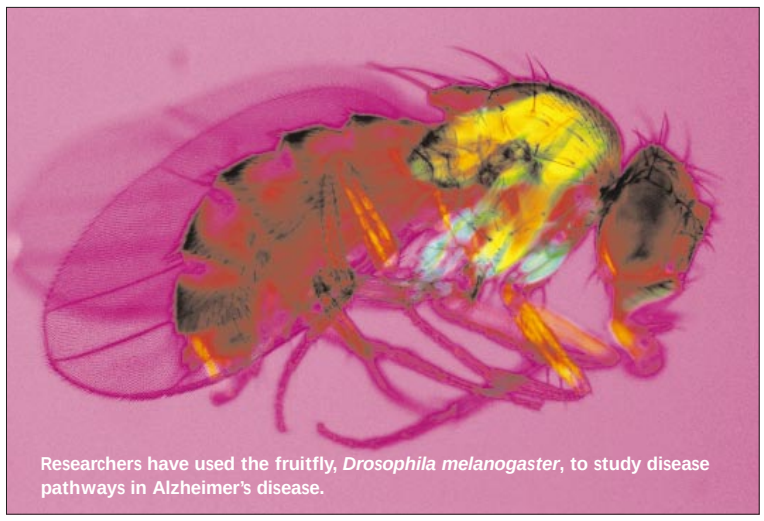

Researchers have used the fruitfly, *Drosophila melanogaster*, to study disease pathways in Alzheimer's disease.

position of the scaffold.

As with all in silico methods, the most practical measure of a methods' success is its ability to retrieve and optimize new lead compounds. Currently there exists a diverse array of approaches to doing this, and increasingly researchers are combining approaches in a multipronged effort to achieve this goal.

The convenience of a single software platform that allows seamless integration of diverse models will become more apparent as workers investigate and explore the potential advantages of these combined models. GEN

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