CURRENT STATUS OF RESEARCH ON TRANSGENIC ANIMAL MODELS FOR ALZHEIMER'S DISEASE

IOANA – MIRUNA BALMUŞ[1*], ALIN CIOBICĂ[1], RADU LEFTER[1], DANIEL TIMOFTE[2]

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Abstract. Each and every pathological visible effect is mostly due to molecular or biochemical reactions on cellular and intracellular level. This was the reason why in the last decade many incurable or poorly understood diseases were at least partially described. This is also the Alzheimer's disease case, extremely aggressive and highly unpredictable. Many studies showed various conclusions used in further research to develop treatments and biochemical therapies in order to slow the degenerative effects or to eradicate the means and motives of this disease. Generally, in order to cure a certain disease, one must surely know the mechanisms of actions involved in its pathology and the start point, also very important. In Alzheimer's disease, there has been studied an entire cohort of hypothesis and by using animal models the researchers reached possible cause variants. Because of the unknown idiopathic Alzheimer's disease etiology, animal models were based on genetic mutations associated with familial Alzheimer, supposing that following events are identical. These genetic models are highly valuable to elucidate the molecular mechanisms which determine the disease's progression, although no animal model can replicate the entire disease symptomatology. For this reason, every animal model type can be used to study one aspect of the disease. For example, transgenic mice overexpressing APP gene exhibit a similar pathology as humans. Age dependence and beta-amyloid accumulation speed was determined also by using transgenic mice. Beta-amyloid plaques discovered in transgenic mice brains were extremely similar to humans ones. Using the same manner of research, it was found a link between beta-amyloid plaques and alfa-synuclein. Plaques coloring method was also developed by various coloring techniques used on histological transgenic mice brain tissue smears. Alzheimer's disease pathology was well studied using transgenic animal models in order to characterize the molecular deficiencies observed in humans. Because of the incapacity of study on human subjects due to ethical reasons and specific human characteristics, several animal models have been developed, which facilitated the study Alzheimer's disease features one at a time.

INTRODUCTION

Alzheimer's disease (AD), nowadays the main cause of dementia, affects over 12 million people worldwide; only 4.5 million of these are in America and there has been predicted to almost triple by 2050 (Herbert *et al.*, 2003; Citron, 2004). Transgenic animal models of AD provide an excellent tool for investigating pathogenic mechanisms and further treatments. Here, we review several animal models used in Alzheimer's disease research.

To begin, we will discuss the pathology of Alzheimer's disease that transgenic models are designed to follow. The first and most common symptom of AD is cognitive function loss (short memory loss). Patients also undergo general cognitive decline including temporal and geographic disorientation, impairment of judgment and problem solving, and deterioration of language abilities (Faber-Langendoen *et al.*, 1988). Behavioral and personality changes also occur until the end stages when troublesome behaviors disappear along with most personality traits (Rubin *et al.*, 1987; Swearer *et al.*, 1988). In the severe stages of the disease, motor complications often develop (Morris *et al.*, 1989; Romanelli *et al.*, 1990) which along with the dementia, leave people completely bedridden and dependent on caregivers.

Recently it has become obvious that AD starts decades prior to its clinical manifestation (Jack *et al.*, 2010; Sperling *et al.*, 2011; Bateman et al., 2012). Exploring the neuropathology of AD in human "pre-clinical" stages is not an easy task. This is the purpose of transgenic animal models – they can shed at least some light on the disease progression factors or on yet undescribed biomarkers or therapeutic targets. This article aims to review transgenic AD animal models currently used, effective or less effective and other relevant studies on the efficiency of transgenesis in AD research.

NEUROPATHOLOGY AND GENETICS

In neuronal tissue, Alzheimer's disease causes an active neuron loss and a synapse number decrease, more often in cortex and sub cortical regions (Wenk *et al.*, 2003). This loss worsens in time leading to mass atrophy of the impaired regions to almost entire inactivation of parietal and temporal lobes, frontal cortex and cingulate gyrus (Braak and Del Tredici, 2012). This degenerative process also occurs in the nuclei of the locus coeruleus. Nuclear resonance studies show that

brain tissue of the Alzheimer patients tends to shrivel like a dried walnut as the patients' behavior worsens (Desikan *et al.*, 2009*;* Moan, 2009).

From a cyto-histopathological point of view, amyloid plaques (βAP) and neurofibrillary tangles (NFT) are highly visible during microscopy examination on Alzheimer's patients' brain tissue smears and sections using specific pigmentation or electronic microscopy. βAP are dense, mainly insoluble β-amyloid (βA) aggregations found particularly in extracellular compartment. NFT are tau protein aggregations formed by aberrant forms' hyperphosphorylation which leads to thread-like aggregates causing disorders in the microtubular system. These are common to most elders, but AD patients exhibit a more increased frequency of aggregate formation, mostly in temporal region. Also, is has been shown that Lewy bodies are quite common to Alzheimer disease but it seems that this is a secondary Alzheimer's effect (Bouras *et al.*, 1994; Kotzbauer *et al*., 2001).

Biochemically speaking, Alzheimer's disease remains to be caused by a characteristic protein missfolding process mainly causing temporal lobe βA accumulation and secondarily in other brain regions (Hashimotot *et al.*, 2003). The small proteins that form the plaques are mostly formed from 39–43 amino acids residues and are the final product of the amyloid precursor protein (APP) pathway. APP is a transmembrane protein which in association with membrane lipoproteins connects the extracellular and intracelullar environment in brain tissue (Priller *et al*., 2006). It seems that APP is involved in neuron maturation, survival and repair. The fact that explains its contribution to Alzheimer's development is yet to be discovered, but it is supposed that the poor APP split during proteolysis leads to smaller fragments than normal (Turner *et al.*, 2005). This may be caused by genetic faults of the APP or of any of the enzymes implicated in the pathway. βA forms extracellular aggregates that break synapse flow. Apart from this, there are other 42 residues fragments highly soluble in the cytosol which activate the casein kinase 2 involved synaptic function inhibition (Ohnishi and Takano, 2004; Hooper, 2005).

At the same time, a fault in the tau protein structure can occur, and cause a high adhesivity leading to tau aggregation with other cellular components. Neurons cytoskeleton is mostly formed by sub cellular structures called microtubules involved in neuron nutrient supplying from neuron soma through axon. Tau protein stabilizes cytoskeleton components structures and it is modulated by a phosphorylation-dephosphorylation mechanism. In Alzheimer, this protein undergoes a succesive phosphorilation serie which bring it in a highly active state protein which gain a supplemental binding activity and therefore supplemental adhesivity. This way NFT are formed and they lead to neuron instability caused by nutrient transport system disturbance. This can occur due to translation faults, posttranslational maturation errors or modulation process slip. The most possible picture is considered to be specific phosphorilase activity disturbance (Hernandez and Avila, 2007).

However, the exact pathogenic mechanism is mostly unknown from a molecular point of view and the way this mechanism is influenced by the βA is yet unclear (Van Broeck *et al*., 2007; Huang and Mucke, 2012). Some studies show that βA accumulation triggers neuronal degeneration, but others show that this accumulation is just another secondary effect (Yankner *et al*., 1990; Chen and Yan, 2006). On the other hand, it has been shown that the βA is an important component of the mitochondrial triggered apoptosis mechanism alongside ionic calcium homeostasis and glucose neuronal usage regulation (Greig *et al*., 2004).

Various research molecular approaches led to conclusions that can offer a starting point for further therapy development which can slow degenerative processes or stop disease trigger. Generally, in order to cure a disease, it is important to know for sure the pathogenic model and the triggering point. Regarding AD, there were many hypothesis of action that led to many possible cause variants. In present, partly because of the current affinity to molecular dimension of life, the biochemistry, genetics and molecular pathogenesis of diseases are mainly studied.

Current research approaches trigger the finding of the real cause. Since 2012, the safety and efficiency of over 400 therapy products has been tested in worldwide clinical trials, but under a fourth of them reached the third phase (Waldemar *et al*., 2007). This was due to the many individual variables as physiological differences, studies say, but the truth is that the predicted effects of the tested products were thought using assumptions not facts. Whether these compounds were not target specific, miss absorbed or metabolized, whether they triggered unpredicted immune reactions, this was possible because of the not fully known pathogenic mechanism. In the end, all clinical trials were suspended because of this and all scientific efforts were directed to disease pathway study.

CURRENT RESEARCH ON ANIMAL MODELS

Current research directions regarding neurodegenerative diseases are mostly targeting diseases' molecular aspects. If until now researchers were desperately trying to find an efficient treatment through pharmaceutical means, now all attention is focused on finding the real cause of the diseases. Because pharmaceuticals only ameliorate the effects of the diseases and mainly of the lack of the real cause, most of the pharmaceuticals' researches were seized.

Many compounds were tested aiming for different pathological features such as βA levels reduction (apomorphine (Lashuel *et al.*, 2002), immunotherapy and APP-based vaccines (Hawkes and McLaurin, 2007; Dodel *et al*., 2010)). The most recent study uses cDNA coated nanoparticles which encodes a monoclonal antibody proven extremely dangerous to

APOEε4 allele carriers, but not as dangerous as another vaccine, ACC-001 (Woodhouse *et al.*, 2007), which can trigger meningoencephalitis development to more than 5% of the patients. The only partially functioning vaccine is bapineuzumab, structurally identical with the natural antibody induced in the βA presence (US NIH, 2008a). There have been tested many other ways of stopping AD development using TNFα receptor fusion proteins (US NIH, 2008b), tau protein phophorilase inhibitors or anti-inflammatory agents but all studies were abandoned (Tobinick *et al.*, 2006) and all attention was focused on animal model research and testing. The most important and developed research branch is the genetic manipulation and transgenic animal models research.

Because the idiopathic Alzheimer's etiology is mostly unknown, animal models research is based on mutation associated with familial Alzheimer's, thinking that the events following triggering mechanism are very similar. These animal models are extremely valuable in determining the molecular mechanism through which the disease evolve, but it has to be said that no animal model can resume all of the disease's features at the same time. Thus every animal model is designed to allow the analysis of only one feature of the disease per model.

Over time, researchers used many animal models based on many mammalian and non-mammalian animals. It is true that the invertebrates could not accurately reproduce AD pathology, but they were a low cost, easy breading alternative to explore cascade mechanisms triggered in AD. As these mechanisms were at least partly discovered, researchers developed more complex transgenic organisms such as mice and rats. Recently, it has been shown that the most eloquent animal model to study is the rat, not the mouse (Do Carmo and Cuello, 2013).

NONRODENT TRANSGENIC MODELS

The most commonly used invertebrate model organisms in the study of brain neurodegeneration are *Drosophila melanogaster* and *Ceanorhabditis elaegans*, an insect and a worm. This is proving that it is not necessarily to study complex animals in order to find responses to complex questions. In fact there is more suitable to study as simpler animal models as we can to find the bases of the mechanisms we are trying to explain. As the evolution and adaptation of all organisms permits us to compare the simplest organisms with the more complex ones, researchers became convinced that it is more suitable to start from more simple animal models.

Therefore a team of researchers found that a homolog of APP is expressed in *Drosophila* and named it amyloid precursor protein-like protein (APPL) (Diagle and Li, 1993). Later they found that the round worm also expresses an APP homolog: the amyloid precursor-like protein 1 (APL-1) (Gunawardena and Goldstein, 2001). Both of these are similar to human APP except the amyloid precursor region. Furthermore, this study showed that expressing human APP in fruit flies and round worms induced neuronal apoptosis dependent upon the presence of the C-terminal and amyloid precursor regions. Studies of APP in *Coenorhabditis elaegans* show that expression of βA in body wall muscles induces progressive paralysis (Link, 1995; Fay *et al.*, 1998).

More than these, both fruit flies and round worms express presenilin homologs, and invertebrate studies have contributed to identification of secretase complex components (Chung and Struhl, 2001, Goutte *et al.*, 2002, Francis *et al*., 2002).

Tau protein has also been studied in both species. Overexpression of wild-type and FTD mutant tau in *Drosophila* results in adult-onset neurodegeneration but without neurofibrillary tangle formation (Wittmann *et al.*, 2001). Overexpression of tau with a tau phophorylation enzyme homolog induces neurofibrillary pathology, though with a different conformation than that seen in human. Coexpression of both tau and APPL in *Drosophila* leads to neuronal dysfunction and disrupted axonal transport. In *C. elaegans*, tau overexpression leads to aggressive pathological changes and behavioral abnormalities (Kraemer *et al.*, 2003).

There is another invertebrate used to study Alzheimer's disease, the sea lamprey, *Petromyzon marinus*. The studies on this fish were important because its central nervous system is characterized by six giant neurons. Microinjections in these were meant to induce chronic tau overexpression and led to rapid degeneration starting with distal dendrites (Hall *et al.*, 2001). Furthermore, with these tests, it has been identified a low molecular weight, lipid-soluble compound that retards the progression of tau-induced degeneration (Hall *et al.*, 2002).

MOUSE TRANSGENIC MODELS

One of the first transgenic models of AD overexpressed amyloid precursor protein in order to reproduce amyloid pathology. This was based on the amyloid hypothesis that predicts that damaged amyloid synthesis pathway leads to Alzheimer-like pathology. Thus, amyloid precursor protein can be processed in two ways - fibrillogenic that leads to plaque accumulation and nonfibrillogenic, with cytosolic location. Familial mutations of APP are associated with accumulation of βA in senile plaques. In order to develop amyloid pathogenesis to mice, many researchers tried to genetically manipulate mice to overexpress human APP under specific promoters.

The most convincing mouse AD model is the PDAPP mouse (Games *et al.*, 1995) that overexpress human APP carrying V717P mutation under the control of PDGFβ promoter 10-fold higher than wild-type normal expressing endogenous APP

mice. It has been shown that they develop Alzheimer-like neuropathologies starting with hippocampus and then extending to cortical and limbic areas with regional specificity as seen in AD pathology (Masliah *et al.*, 1996; Irizarry *et al.*, 1997). Behavioral tests certified that amyloid pathology is age-dependent as it has been shown in human. More than that, it has been shown that the amyloid pathology in PDAPP mice is similar to that observed in AD even in ultrastructural details. Although entorhinal cortex, CA1, or cingulate cortex neuron loss was not observed (Irizarry *et al.*, 1997), the pattern of neuron loss was observed mimicking human AD patterns (Urbanc *et al.*, 2002).

Tg2576 mice line was another mice AD model that overexpressed APP double Swedish mutation (K670N and M671L) cDNA controlled by hamster prion protein promoter (Hsiao *et al.*, 1996). The APP production was lower than PDAPP mice, but the effects were very similar to those seen in AD. During behavioral tests it has been observed that some functional disruptions may underlie some of the observed memory deficits.

APP23 mice, APP cDNA with Swedish mutation under control of a murine promoter carriers, develop both amyloid plaques and cerebral amyloid angiopathy starting earlier that the other transgenic mice. Also, they develop memory deficits as assessed by behavioral tests (Lalonde *et al.*, 2002; Kelly *et al.*, 2003; Van Dam *et al.*, 2003).

There have been other attempts to genetically manipulate mice for AD study but they do not develop plaques until 18 months (line APP Swe C3-3) (Borchelt *et al.*, 1996; Borchelt et al., 1997), or trigger premature death (TgCRND8 mouse model) (Christi et al., 2001; Dudal *et al.*, 2004).

Besides APP mutations, there has been shown that presenilin genes mutations can trigger familial AD, probably by altering the processing of APP in favor of βA production to generate animal models. Overexpression of either M146L or M146V mutations under the PDGFβ promoter causes a selective increase in βA_{42} production (Duff *et al.*, 1996; St George-Hyslop, 2000).

To model the NFT pathology, researchers have been developed tau transgenic mice. The tau protein located in axons, appears in AD hyperphosphorylated in cell bodies and dendrites. Mutations of MAPT gene cause FTDP-17 syndrome (frontotemporal dementia and parkinsonism linked to chromosome 17) by reducing tau's ability to bind to microtubules or splice the tenth exon increasing aberrant isoforms (containing 4 microtubule-binding domains) (Hutton *et al.*, 1998).

One of the first transgenic tau animal models was transgenic mice expressing wild-type 4 repeat human tau. Hyperphosphorylation of tau and aberrant localization, but not NFT, were observed in these mice (Gotz *et al.*, 1995). Other studies showed that regardless the gene promoter used, the transgenic mice developed hyperphosphorylation of tau and pathologies along association neurons' axons in central and spinal cord, but never NFT (Spittaels *et al.*, 1999; Gotz *et al.*, 2000; Probst *et al.*, 2000). These showed that overexpression of human wild-type tau is not sufficient to induce NFT. Rare NFT were observed in transgenic mice overexpressing wild-type human 3 repeat tau alongside hyperphosphorylation of tau in the hippocampus, amygdala, and entorhinal cortex, though very late in life.

After MAPT mutations discovery, many groups begun to develop animal models using them. Thus NFT formation was observed in P301L mutation mice carriers (JNPL3 line) in spinal cord, brainstem, cerebellum, diencephalon, and basal telencephalon (Lewis *et al.*, 2000; Arendash *et al.*, 2004)[67,68]. New P301L tau transgenic mice that can demonstrate progressive neurofibrillary pathology and neuronal loss in AD has recently been developed (Gotz *et al.*, 2001). In P301S transgenic mice were observed high expression of tau in spinal cord, brainstem, hippocampus, and neocortex, death of half of the motor neurons in spinal cord and hyperphosphorylation of tau, but no NFT (Santa Cruz *et al.*, 2003).

There have been developed many transgenic mice based on interactions between APP, tau protein, βA, presenilins and other risk factor genes that could explain why overexpression or mutations are not always enough for AD triggering. For example, the most common association demonstred via transgenic mice was the association between apolipoprotein E ε4 allele presence with increasing risk of AD seen in amyloid pathology context. Recent research show that ApoE may affect tau phosphorylation and accumulation processes. Overexpression of ApoEε4 and ApoEε3 induces aggregation of phosphorylated tau in regions vulnerable in AD of the brain of the transgenic mice (Brecht *et al.*, 2004). Changing expression promoter, they show that ApoEε4 may be neuron-specific (Brecht *et al.*, 2004). Other studies used conformational change knock down or up constructs showing that tau processing enzymes mught also be involved in AD pathology and NFT formation (Ahlijanian *et al.*, 2000; Lucas *et al.*, 2001)

TRANSGENIC MICE DRAWBACKS

Using transgenic mice, many groups discovered the AD pathology features. Beginning with βA role, inflammatory responses, APP and presenilins functions, and ending with posttranslational processing of tau protein, all of which have been studied using different transgenic mice models. However, the hallmark of AD – massive or selective neuron death – has not been exhibited by any mice model published so far, with one exception (Santa Cruz *et al.*, 2003). It seems that massive neuron loss is a human specific feature of AD due to longer life or a more vulnerable brain tissue. More than that, the βA and NFT distribution in mice was never exactly reproduce to that in human. And, to be more accurate, recombinant DNA used in transgenic mice was under an exogenous promoter, in other words, there weren't any regulating native sequences in the transgenic construct (Aronov *et al.*, 1999). Also, in order to mimic the exact human

conditions, there must be done a transgenic construct that can express all of the six tau isoforms. This can be done by constructing an entire tau human gene expressing model but it has been observed that this is correlated with a three or four-fold increase in tau mice brain concentration and absence of murine tau expressing, and in absence of a tau mutation, these mice did not develop any aberrant phenotype (Duff *et al.*, 2000).
There has been made a correlation between cognitive performance and tau and βA pathology in behavioral studies and the possibility of βA plaques removal in immunological studies, but the precise mechanism and relationship between removal and memory function is not fully understood.
Despite all the limitations mentioned, transgenic mice have been extremely valuable in AD research (Hartley *et al*., 1999). However, the production of transgenic mice takes time and resources and it has been proved an inefficient task. This is true for single transgenic mice, but even more pronounced in two or three transgenic lines. However, the transgenic strategies are never abandoned but improved. Given that there are many species in the rodent family, it seems that there is another valuable rodent available for research – the rat.

THE RAT – THE NEW AD MODEL

Along time, mice have always been preferred in transgenesis studies in spite of the rats mainly because of the size and needs. More than that, rat's zygotes are more demanding regarding the transgene injection perform (Charreau *et al.*, 2004) and success. The gene replacement or loss of function mutation strategies (knock in/knock out) are hampered by the difficult to obtain rat embryonic cells. These few drawbacks are insignificant compared to the numerous advantages.
Firstly, between rat and mouse, the rat is physiologically, genetically and morphologically closer to humans than mice (Lin, 1995; Jacob and Kwitek, 2002; Gibbs *et al*., 2004). Because it is larger, the intraventricular administration of different drugs and cerebrospinal fluid sampling is easier (Tesson *et al.*, 2005).
Biochemically speaking, rat genome contains informations for the expression of six tau isoforms, as human genome does too (Hanes *et al*., 2009), although there is a slightly difference between major isoforms ratio. More than that, both human and rat share almost 73% of the ApoE protein amino acid sequence (McLean *et al*., 1983; Rajavashisth *et al.*, 1985), and the rat ApoE features are similar to ApoE3 in human (Tran *et al.*, 2013).
Behavioral speaking, it is well known that the rats are behaviorally well characterized. It has been shown that they have finer and more accurate motor coordination than mice and their behavioral display is more observable than in mice. They express social behavior and age-related aggression (Whishaw *et al.*, 2001). Since rat's life environment is a complex one, combining both terrestrial and aquatic elements, behavioral test on land or water is more facile (Whishaw *et al*., 2001). These differences are possible because of the post-natal brain development occurred both in rats and humans, but not in mice.
Thus rat models should permit a more complex characterization of behavior, cognition and pathology levels. More than that, they are more valuable tool in drug and therapies testing. Based on all these advantages, rats are increasingly used to mimic pathological hallmarks of Alzheimer's disease (Taravini *et al*., 2011; Kitamura *et al*., 2011; Nuber *et al.*, 2013). What is the most important is that there have been developed certain transgenic rats models that are a more accurate representation of the human pathology model.

CONCLUSIONS

Alzheimer's disease pathology was well studied using transgenic animal models in order to characterize the molecular deficiencies observed in humans. Because of the incapacity of study on human subjects due to ethical reasons and specific human characteristics, several animal models have been developed, which facilitated the study Alzheimer's disease features one at a time. Over time, researchers used invertebrates such as fruit fly and common round worm and vertebrates such as the sea lamprey or mammals to mimic pathologies of interest, but it seems that a more valuable tool have been discovered for genetic manipulation and study – the rat. It has been shown that the rat posses certain features that make him closer to human than the previously used animal models.

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1 Faculty of Biology, University of “Alexandru Ioan Cuza” Iaşi, Romania,
2 University of Medicine and Pharmacy "Gr. T. Popa" Iași.
***** balmus.ioanamiruna@yahoo.com.