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Review Article

Different Therapeutic Approaches Against Alzheimer's Disease and Usefulness of Drosophila as AD Model

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Abstract

Alzheimer's disease (AD) is a common human neurodegenerative disorder. It is basically occurs in elderly people (age more than 60). Till date very less understanding about causes of AD that's why there is no cure of the same occurs. People are focusing to find out suitable therapeutic potential for the treatment of AD by using different approaches. People are using different model system for the study and validation of different synthetic as well as herbal compounds against treatment of AD. Even AD etiology is not well known yet. So this review basically discusses the fundamental about AD and different therapeutic approaches for treatment of AD using Drosophila model system.

***Keywords**: Alzheimer's disease; Drosophila model system; Neurodegenerative disorder*

Introduction

Alzheimer's disease is a neurodegenerative disorder [1]. It is the most common form of dementia [2,3]. AD is characterized by the memory loss, irritability and mood swings[4]. It has no any cure till today. It was first described by German psychiatrist Alos Alzheimer in 1906[4].AD is basically associated with aging[5].The cause of the AD is not well understood. There are basically two hallmark of the AD; one is extracellular deposition of senile plaques and other intracellular formation neurofibrillary tangles (NFT) in the brain [6, 7].The treatments available at present only help with the symptoms of the disease but do not stop the progression of the disease. So the different therapeutic approaches in search of suitable drug target for the treatment of AD are demanding. Nowadays people are using different synthetic as well as herbal compounds for the treatment of AD. Earlier studies from our group reported the anti-Aβ activity of a novel flavonoid derivative [8] as well as showed neuroprotective role of a novel copper chelator against copper mediated Aβ toxicity [9]. Furthermore, Zhang et. al. showed dual effect of a synthetic molecule having anti-Aβ as well as copper chelation property [10].Several other groups are also focusing to find out the suitable therapeutic potential against AD using anti-Aβ approaches [11-14].In this review, we have discussed different therapeutic approaches used for the treatment of AD using drosophila model system with brief description of other model system used in AD study.

The disease is characterized into four stages:

Pre-dementia: It is related to aging and stress. Memory-loss and apathy are commonly found in this stage [15,16].

Early: Language problem appears. Patient feel problem in planning [16]

Moderate: It is characterized by wandering, irritability and labile effect, leading to crying, aggression and resistant towards care giver [16]

Advanced: In this last stage, person becomes completely dependent on the care giver. Complete loss of speech and ability to feed by themselves occurs. Extreme apathy and exhaustion are commonly found [16].

Cause: The exact cause of Alzheimer's is still unknown [17]. Several hypotheses are given to explain the cause. Some prominent hypotheses are: Cholinergic hypothesis, tau hypothesis, amyloid hypothesis, apoE gene related hypothesis and age-related myelin breakdown in the brain [18,19,20,21,and22].But amyloidal hypothesis is the most accepted one [23, 24, 25].

Amyloid hypothesis

In 1991, this hypothesis postulated that amyloid beta deposits were the main cause of the disease [26].It was supported by the postulate that gene for amyloid beta precursor protein (APP) is located on chromosome 21 and APOE4 which is the major genetic risk factor leads to excess synthesis of β-amyloid (Aβ) in the brain [27].Researchers have assumed that Aβ oligo mers are the primary pathogenic form of Aβ. These toxic Aβ oligomers are also referred to as amyloid derived-diffusible ligands (ADDLs) which bind to the surface receptors on neurons and alter the structure of the synapse, by disrupting the neuronal communication [28].In 2009the theory was updated, which suggests that some age-related processes may also cause the neuronal withering. N-APP, a fragment of APP from the N-terminus, is cleaved from the APP peptide by one of the same enzyme which cleaves beta amyloid from the APP peptide, triggers the self-destructing pathway by binding to the neuronal receptor called death receptor6 (DR6).DR6 is expressed highly in the human brain region affected by Alzheimer's disease. So it is possible that the N-APP/DR6 pathway may be blocked by the aging brain to cause the defect [29,30].

Biochemistry of AD

AD is considered as protein mis folding disease, caused by the accumulation of mis folded Aβ and tau proteins in the brain[31]. Plaques found are made up of small peptides of 39-43 amino acids in the brain known as beta-amyloid [32,33]. Aβ is the fragment of larger peptide APP, which is the trans-membrane protein that is found in the neuron's membrane [34,35].During AD, APP undergoes proteolytic processing by two different pathways namely: amyloidgenic pathway by the action of β and γ-secretase and non-amyloidgeinc pathway by α- and γ-secretase [36, 37, and 38]. In the amyloidogenic pathway, APP undergoes cleavage by β-secretase, encoded by BACE gene. This cleavage produces a soluble extracellular fragment of APP (sAPPβ) and a membrane spanning C-terminal fragment (βCTF/C99).The γ-secretase then cleaves βCTF to produce Aβ peptides and the APP intracellular domain (AICD).Aβ peptides of variety of lengths are produced but Aβ40 and Aβ42 are the major is forms produced in the CNS.Aβ42 is subjected to more oligomerization and is more neurotoxic [39-42]. In non-amyloidogenic pathway, α-secretase cleaves APP to produce a soluble extracellular/luminal fragment of APP (sAPPPα) and a membrane spanning C-terminal fragment (αCTF/C83). Againγ-secretase complex cleaves αCTF to produce the P3 peptide and AICD[43].

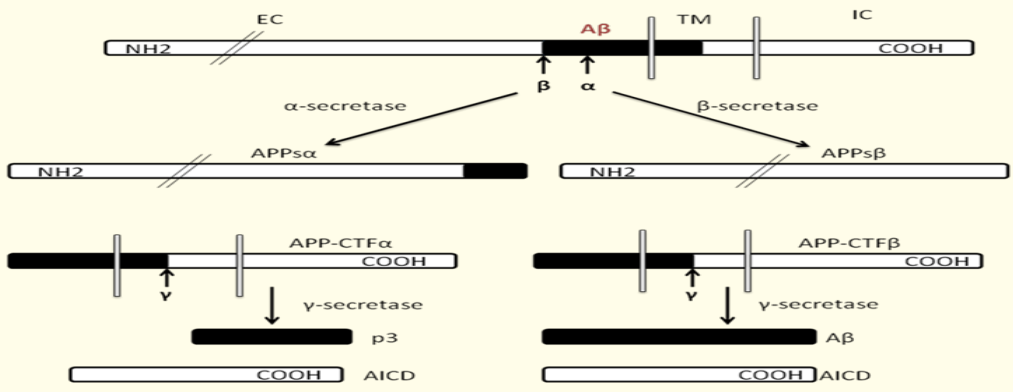

***Figure 1** : Showing both the amyloidogenic and non-amyloidogenic pathway of Aβ proteolytic cleavage. Adapted from Annaert and De Strooper (2002).*

Study of Alzheimer's disease using different model organisms:

Mice

Use of mice for AD research is very popular. In a study using transgenic mice as model organism for Alzheimer's disease, it was found that active immunization with the amyloid β(Aβ)peptide decrease Aβ deposition in brain and certain peripherally administered anti–Aβ antibodies also show similar effect. On finding the cause of Aβ clearance and metabolism ,it was found that a monoclonal antibody (m266) directed against the central domain of Aβ was able to bind and completely hinder plasma Aβ. On peripheral administration of m266 to PDAPP transgenic mice, in which Aβ is generated specifically within the central nervous system (CNS),result in a rapid 1000-fold increase in plasma Aβ, due to which Aβ equilibrium changes between the CNS and plasma. The peripheral administration of m266 in the transgenic mice effectively reduces the Aβ deposition, but m266 did not bind to Aβ deposits in the brain. Thus,m266 reduces the brain Aβ burden by altering CNS and plasma Aβ clearance[44,45,46].The "central domain" anti-Aβ antibody, monoclonal antibody 266 (m266), rapidly sequesters all plasma Aβ present in PDAPP mice and causes a large accumulation of centrally derived Aβ in the plasma. Peripherally administered m266 also causes rapid increases in CSF Aβ, part of which does not appear due to entry of the antibody into the CNS. Finally, chronic parenteral treatment with m266 results in marked suppression of Aβ deposition in brain, suggesting that certain anti-Aβ antibodies suppress AD-like pathology by altering Aβ clearance from CNS to plasma [47].

Caenorhabditis elegans

In C. Elegans the study of Alzheimer's disease was done by studying the effect of oxidative stress and relation of Aβ fibril formation with the disorder. A temperature inducible Aβ expression system was employed in C. elegans to create a transgenic strain of worms,CL4176,in which Aβ (1-42) is expressed at a temperature of 23^0C.The transgenic strain helps in studying the relation between Aβ expression, oxidative stress, and Aβ fibril formation.CL4176 were under high oxidative stress as compared to the control animal[48,49,and 50]. The increased oxidative stress occurred in the absence of Aβ fibril formation, showing that the toxic component in Aβ toxicity is pre-fibrillar Aβ and not the Aβ fibril. The elevated oxidative stress increases the protein carbamoyl formation which inturn increases the Aβ expression but in the absence of the Aβ fibril formation, suggesting that pre-fibrillar Aβ is the Aβ toxic species. This resulted in a hypothesis that fibrils are not required for neurotoxicity[51, 52, 53, and 54].Aβ toxicity involves a mulitimer, which serve as an intermediate in fibril formation [55].

Zebra fish

Alzheimer's disease is also studied using zebra fish as the model organism .Zebra fish is used frequently because of the higher production of eggs per spawning, transparent embryos and external development. Zebra fish contain most genes similar in function to genes known to involve in AD. It has two genes similar to human APP, app a and app b [56]. Orthologs of the β-secretase and γ-secretase complexes are found in it and expressed in CNS [57, 58, 59, 60].

To understand how the microtubule-associated protein tau (MAPT) contributes to tau pathology and how tau form pathogenic neurofibrillary tangles, a zebra fish model transiently expressing mutant human tau has been reported [61]. In this study, human tau carrying mutations at sites associated with hereditary dementias was fused to GFP under the control of the zebra fish pan-neural–specific GATA-2 promoter. GFP-positive neurons were found in the brain, retina, and spinal cord. In the brain, there was evidence which was significant for AD-associated cytoskeletal pathology that includes disruption of tau trafficking and cytoskeletal filaments in the axon, accumulation of tau in the cell near the axon, and hyper phosphorylated tau [62, 63, 64].

In an another study using zebra fish model to study methods of treating Alzheimer's disease is by diverting APP away from the late endosomal pathway by a SORL1-dependent switch, which sequesters APP into recycling endosomes, preventing the formation of Aβ[65]. This genetic association between AD and SORL1 expression is a result of single nucleotide polymorphisms (SNPs) found within the SORL1 gene[66]. It has been demonstrated that SORL1 binds directly to APP and differentially regulates the sorting of APP into the late endosomal pathway leading to the production of cytotoxic Aβ or into the retromer recycling pathway, sequestering APP from the β- and γ-secretases. The overexpression of SORL1 in HEK cells reduces Aβ production by 82%, likely by diverting APP into the retromer recycling pathway [67,68].

Drosophila as model organism

Drosophila melanogester belongs to the family Drosophilidae, of the order Diptera. It is commonly known as fruitfly.

Scientific Classification

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Diptera

Family: Drosophilidae

Genus: Drosophila

Subgenus: Sophophora

Species group: melanogaster group

Species subgroup: melanogaster subgroup

Species complex: melanogaster

Complex Species: D. melanogaster

Physical appearance:

Wild type fruit flies have brick red eyes, are yellow-brown in colour, and have black rings across their abdomen. They exhibit sexual dimorphism: females are about 2.5 mm long while males are slightly smaller and back of their bodies are darker. Males can be easily distinguished from females on the basis of colour difference, distinct black patch at the abdomen and also the presence of sex combs on four legs. Furthermore, males have a cluster of spiky hairs (claspers) surrounding the reproducing parts used to attach to the female during mating.

Drosophila is the most commonly used model organism in present days because of the following reasons: It has large reproducing capacity, which varies widely according to the species. It can be easily cultured in lab and culture requires simple B. O. D and uses little space even when using large cultures and the overall cost is low. It is small and their morphology is easy to identify once they are anesthetized (usually with ether, carbon dioxide). It has a short generation time (about 10 days at room temperature) so several generations can be studied within a few weeks.

Males and females are readily distinguished and virgin females are easily isolated, facilitating genetic crossings. Males do not show meiotic recombination, facilitating genetic studies

Recessive lethal balancer chromosomes carrying visible genetic markers which can be used to keep stocks of lethal alleles in a heterozygous state without recombination due to multiple inversions in the balancer. Its complete genome is sequenced[69,]

Similarity to human

About 75% of known human disease genes is homologues to the genome of the fruit flies and 50% of fly protein sequences are found to be homologous to mammalian protein sequences. An online database called Homophila is available to search for human disease gene homologues in flies and vice versa. Drosophila is being used as a genetic model for several human diseases including the neurodegenerative disorders Parkinson's, Huntington's, Spinocerebellartaxia and Alzheimer's disease. The fly is also being used to study mechanisms underlying aging and oxidative stress, immunity, diabetes, and cancer, as well as drug abuse[70, 71].

Homology of Drosophila with human beings in Alzheimer's disease

Drosophila is very much homologous to a human that's why it is being used to study human diseases. It has similar fundamental aspects of cell biology, including regulation of gene expression, membrane trafficking, the cytoskeleton neuronal connectivity, synaptogenesis, cell signaling and cell death. Many genes and pathways found in flies are also identified in mammals.

The amyloid plaques are considered to be the key feature of the AD. The main component of these plaques is the Aβ peptide, which is derived from membrane, bound amyloid precursor protein (APP).Dominant mutations in APP or Psn 1and 2 cause early onset of AD. Homologues of the APP and presenilin are found in drosophila. The fly homolog of APP is Appl. [72,73].The β and γ-secretases are respon-

sible for the proteolytic processing of APP to produce Aβ peptides. Presenilin is one of the components of the γ-secretase complex. Its homolog identified in the flies is Psn which is required for the proteolytic processing of Notch [74].

The second key responsible for the disease is neurofibrillary tangles. Tau is a microtubule-associated protein, and its interaction with microtubules is negatively regulated by phosphorylation of sites in or near its microtubule binding repeats. Taupathy is responsible for the development of the neurofibrillary tangles. During taupathy, phosphorylation of tau protein and its microtubule binding is aberrantly regulated. Drosophila with taupathy has also been identified. The fly tau homolog has been cloned and characterized. Williams and co-worker found that over expression of human tau in sensory neurons produced a number of abnormal morphologic effects, including axonal loss and swelling. Sensory neurons expressing tau underwent axonal degeneration. Recently, these authors showed that misexpression of a constitutively active form of the tau kinase glycogen synthase kinase (GSK)-3β enhanced specific axonal transport defects and motor behavior caused by tau [75].

Phenotypes and surrogate marker used to measure pathology in fly models of AD

The most widely used behavioral assays that are employed are Pavlovian conditioning tests of memory and learning [76, 77] and loco motor assays [76, 78, 7980]. In flies, loco motor assays such as the climbing test are popular because they need little equipment and they measure a clear phenotype. The loco motor assay is performed by placing the flies at the bottom of a tall cylinder and allowing them for a specified time to climb before the number of flies at the top and bottom of the tube are counted and the ratio is expressed as a performance index [80, 81]. It was found that the fractional increase in median longevity as compared to control flies provides a parameter that has validity as a comparison between, as well as within, particular experiments [82].By tracking flies we are able to calculate which parameters, such as maximal, mean and median velocity, best describe and distinguish control flies from those affected by model AD pathology. This approach allows detecting subtle changes in loco motor behaviour that characterize the early stages of neuronal dysfunction.

Another commonly used marker is the rough eye phenotype which is also easily recognized and has been particularly useful in the fly taupathy models, where human tau expression in the retina yields adult flies with rough, shrunken eyes [82, 83]. The use of genetic screens in the fly models of AD has been focused on understanding the biological pathways by which deregulated Aβ and tau production and aggregation might cause neuronal dysfunction and death. Figure 2 showing the pattern of rough eye phenotype in modeling Aβ in drosophila.

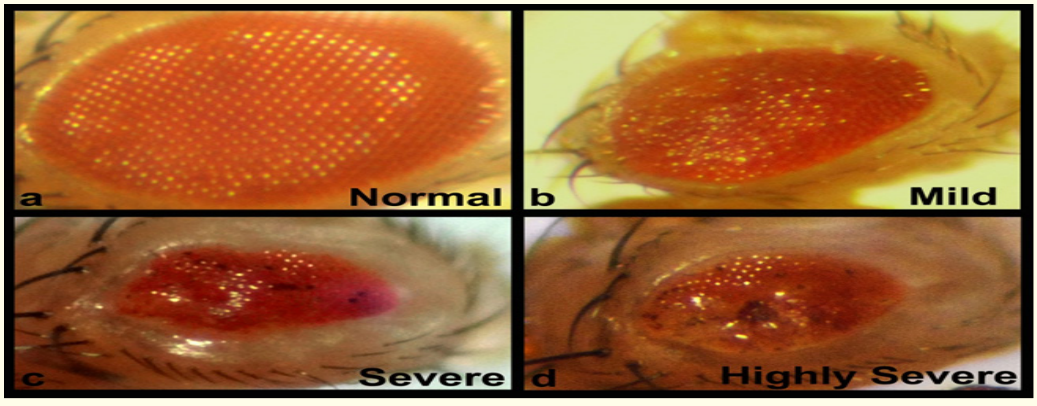

Figure 2: The normal compound eye (a) exhibits a regular array of corneal lenses (ommatidia) that are disrupted when toxic proteins are expressed during development. The assessment of eye roughness allows the investigator to look for disruption in eye morphology when Aβ express in eye tissue and different phenotype were observed like mild (b), severe (c) and highly severe (d) eye phenotype.

Earlier studies of Alzheimer's Disease using Drosophila as model organism

Drosophila models was used to study the relation between mutant presenilin causing earlier onset of Alzheimer's disease in humans and presenilin in Caenorhabditis elegans which facilitate notch receptor signaling. Drosophila presenilin homologue of human presenilin was isolated and the spatial and temporal distribution of the encoded protein was distributed as well as its localization relative to fly notch protein. It was found that Presenilin is widely expressed throughout oogenesis, embryogenesis, and imaginal development, and generally accumulates at comparable levels in neuronal and non-neuronal tissues. Double immuno labeling with Notch antibodies revealed that Presenilin and Notch are co-expressed in many tissues throughout Drosophila development and display partially overlapping sub cellular localizations, supporting a possible functional link between Presenilin and Notch [84].

In other, the study was done in relation to therapeutic of AD that decrease in presenilin function in Drosophila mealnogester increases the level of apoptosis in developing tissues. Moreover, when presenilin is expressed at an elevated level exerts dominant negative effect. In Drosophila S2 cells, Psn over expression leads to reduced Notch receptor synthesis. A genetic model was described in which the apoptotic activities of wild-type and mutant presenilins can be assessed, and was found that Alzheimer's disease linked mutant presenilins are less effective at inducing apoptosis than wild-type presenilins [85].

Therapeutic Approach

Nowadays people are using different therapeutic approaches for the treatment of AD by targeting Aβ, Tau, different secretases and other candidates involved in AD pathology. The direct binding of Aβ and apolipoprotein E(apoE) is an important factor in both Aβ clearance and its deposition in the brain cells[86,87,88, and 89].As a potential therapeutic target, the blocking of the apoE/Aβ interaction was made by developing Aβ12-T28P, which is permeable to blood-brain-barrier, nontoxic, and non-fibrillogenic synthetic peptide homologous to the apoE binding site on the full length Aβ[90, 91].Aβ12-T28P binds with high affinity to apoE, preventing its binding to Aβ, but has no direct effect on Aβ aggregation.Aβ12-28P shows a strong pharmacological effect in vivo[92, 93].Its systematic administration resulted in reduction in Aβ plaques and cerebral amyloid angiopathy burden and a reduction in total level of Aβ in the brain of AD transgenic mice models [94, 95]. The treatment does not have effect on soluble Aβ fraction or Aβ oligomers, indicating that inhibition of Aβ/apoE interaction has effect on Aβ clearance over deposition and also favor the conditions which inhibit the formation of toxic oligomers. The treatment of Aβ12-28P prevents a memory defect in transgenic animals [96].

In another approach a naturally occurring polyphenol known as resveratrol(tans-3,4,5-trihydroxystilbene)[97, 98, 99],mainly found in grapes and red wine, remarkably lowers the level of secreted and intracellular amyloid Aβ peptides produced from different cell lines. Resveratrol does not inhibit Aβ production, because it has no effect on the Aβ producing enzymes β and γ-secretases, but promotes intracellular degradation of Aβ by the help of mechanism involving the proteasome. Therefore these findings demonstrate a proteasome-dependent anti-amyloidogenic activity of resveratrol and suggest that it has a therapeutic potential in Alzheimer's disease [100].

In another study it is aimed to use inhibitors of β and γ-secretase to inhibit the production of Aβ as an important therapeutic approach for the treatment of Alzheimer's disease. Several simple assays are applied in high throughput screening of candidate β and γ-secretase inhibitors for preventing Aβ production [101].

The γ-secretase reporter assays using the APP C99–GVP constructs have been reported [102]. The GVP construct consists of the Gal 4 DNA-binding/VP16 transactivation (GVP) domain fused to the C99 region of APP, which serves as a substrate for γ-secretasein vivo. This assay offers several advantages for detection of AICD which can be quantified because the presence of the GVP domain reduces cyto solic degradation of the AICD fragment. This assay has the possibility of being useful in high throughput screening of candidate γ-secretase inhibitors for APP [103].C99-rtTA chimeric assay system was also similarly developed to monitor α-secretase activity in living cells in real time [104].

Similarly chimeric constructs that possess alkaline phosphates (AP) fused to APP are widely used for studying APP processing and BACE activity. SEAP–APP is a very sensitive indicator of BACE cleavage. After co-transfection of the SEAP–APP construct with a β-gal reporter construct and target, SEAP activity was measured in the medium and normalized to β-gal activity. Because wild-type APP is a rather

poor substrate for BACE-1, substrates were developed based on the sequence of the mutant of APP, an FAD mutation that enhances Aβ production. It enabled development of a sensitive biochemical assay in which the BACE-1 concentration can be as low as 100 pM. Moreover, this assay and substrate are applicable for counter screening BACE-2 and cathepsin D [105].

Pathophysiology of Alzheimer's disease and the targets of drug action:

Drugs for AD: Drugs can be categorized in two categories such as approved drugs whose role is known and experimental drugs whose role is yet be known.

Approved Drugs-Cholinesterase inhibitors
-NMDA antagonist

Experimental Drugs-Nicotinic Agonist Receptor, Antioxidants, PPAR gamma Agonist, Gamma secretase inhibitors, Statins, 5HT-6 Agonist [106].

Cholinesterase inhibitors

AChEI is approved for the treatment of mild to moderate AD.Four ACHEI is approved by FDA. They are Tacrine, Donepezil, Rivastigmine and Galantamine. Introduction of tacrine was a significant and in the treatment of AD. But adverse effects were reported like hepatotoxicity and cholinergic effects.

Then tacrine has been replaced by Donepezil. Donepezil is a reversible inhibitor of AChEI with a long plasma half-life of 70 h. It is not hepatotoxic Donepezil is found to bring significant benefit in patients receiving 5 and 10 mg Donepezil. The U.S. Food and Drug Administration have approved the first generic versions of Aricept (donepezil hydrochloride) orally disintegrating tablets (www.fda.gov). Recently, the FDA has also approved 23 mg extended release tablet Donepezil for the treatment of moderate to severe Alzheimer's disease (www.clinicaltrials.gov)

The US FDA has also approved Rivastigmine capsules and the Rivastigmine patch for the treatment of mild to moderate dementia of the Alzheimer disease (www.fda.gov)

Galantamine is a reversible and selective AChEI having 50 times more selective for human acetylcholinesterase than for human butyrylcholinesterase. Galantamine also acts as a nicotinic receptor agonist in the brain. Galantamine was found to be efficient.

NMDA receptor Antagonist

Glutamate is an excitatory neurotransmitter and acts on a variety of receptors. NMDA is one such receptor. NMDA receptor on activation causes potentiatial neuronal activity, but in Alzheimer's disease, excessive glutamatergic excitotoxicity causes apoptotic cell death and defects in cognition and memory. While, a NMDA receptor antagonist is recently approved by FDA for the treatment of moderate to severe Alzheimer's disease, as it is found to interfere with the glutamate excitotoxicity[106].

Use of UAS-Gal4 system in the study of Alzheimer's disease using Drosophila as model organism:

GAL4 system in Drosophila:

Gal4/UAS is an important tool for targeted gene expression in Drosophila. This system has also helped Drosophila attract attention from the biotechnology industry as a viable means to investigate the function of genes implicated in a wide variety of medically and economically important processes.

GAL4 encodes a protein of 881 amino acids, identified in the yeast Saccharomyces cerevisae as a regulator of genes (GAL10 and GAL1) induced by galactose. In a number of notable studies on transcriptional regulation, the DNA binding and transcriptional activation functions of GAL4 were identified. It regulates the transcription of the divergently transcribed GAL10 and GAL1 genes by directly binding to four related base pair sites located between these loci. These sites define an Upstream Activating Sequence (UAS) element, analogous to an enhancer element defined in multi cellular eukaryotes essential for the transcriptional activation of these GAL4-regulated genes. The DNA binding activity of Gal4 maps to the first 74 residues, while its transcriptional activation function maps to two regions, residues

148-196 and 768-881.Gal4 expression was capable of stimulating transcription of a reporter gene under UAS control in Drosophila[107].

Conclusion:

Alzheimer's disease is a common human neurodegenerative disease with a complex and long asymptomatic period prior to place able clinical dementia. It has been well established that Aβ aggregation plays a significant role in neurodegenerative disorders such as AD, which may result in neuronal cell death. Therefore, different approaches utilizinganti- Aβ therapy preventing or reducing Aβ mediated neurotoxicity may provide therapeutic potential towards AD treatment. Most anti-Aβ drugs show a positive effect for AD prevention in cell lines and animal models but are less effective when it comes to clinical trials in humans; clinical trials for AD treatment by the use of different anti-Aβ drugs are still in their inception. Most of the known drugs are limited in their ability to cross the blood-brain barrier (BBB). Therefore, development of smaller molecules that would easily pass through BBB offer more promises for anti-Aβ drug development for AD treatment. Additionally, it is imperative that future trials utilise combinations of anti-Aβ drugs along with tau targeted drugs, rather than a single one. This might help to find out suitable therapeutic potential against treatment of AD. Further studies are required to determine if different synthetic as well as herbal anti-Aβ drugs may significantly decrease the risk of AD development or slow the progression of the disease for AD patients.

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