August 1, 2017
TRANS GENIC INC.
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TRANS GENIC Group to Launch Non-Clinical Test Service Using Mice Models of Central Nervous System Diseases

TRANS GENIC INC. (CEO: Kenji Fukunaga, Fukuoka-city, Fukuoka, Japan) hereby announces that, TRANS GENIC Group will launch new non-clinical test service utilizing mice models of central nervous system diseases.

[Overview]

The core business of TRANS GENIC has been the production service of genetically engineered mice from the time the company was established. Now, TRANS GENIC provides wide variety of mice-related services, including mice production, preservation and reproduction of mice strains, and distribution of mice models. On the other hand, New Drug Research Center, Inc., the affiliated company of TRANS GENIC Group, provides clinical testing service of food, such as non-clinical study at GLP-compliant laboratories and GCP-conforming bioequivalence test, using its wide experience and know-how.

Taking advantage of each characteristic, both companies will launch new testing service mutually to evaluate the efficiency of central nervous system agents using mice models of central nervous system diseases, such as Alzheimer's disease, dementia, and neuropsychiatric disorders.

<Mice Models>

	Strains	Mutation	
		Construct	Promoter
Alzheimer's disease	APPosk-Tg	Human APP E693Δ	Mouse prion promoter
Dementia	SJLB	Human N279K TAU	Mouse prion promoter
neuropsychiatric disorders	proBDNF-KI	Mutant proBDNF	(endogenous)

※Histological analysis and biochemical analysis are also available. Please contact us for more information.

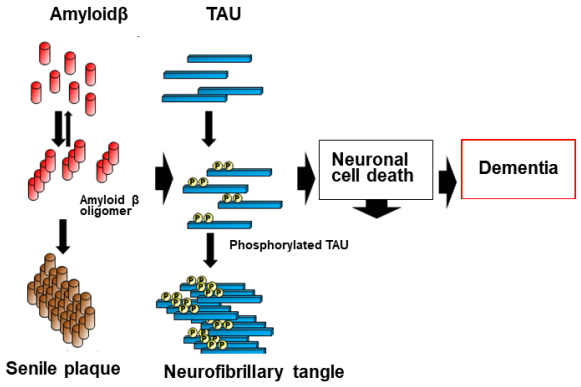
[Reference]

Mice model of Alzheimer's disease

Alzheimer's disease ("AD") is a progressive neurodegenerative disease accompanied by the decline in cognitive function. The pathological change in brain includes the accumulation of insoluble aggregate consisting of amyloid β ("Aβ"), called senile plaque, the accumulation of insoluble aggregate consisting of TAU protein, called neurofibrillary tangle ("NFT"), and brain atrophy.

"Amyloid hypothesis" is known as the AD pathogenesis as indicated below:

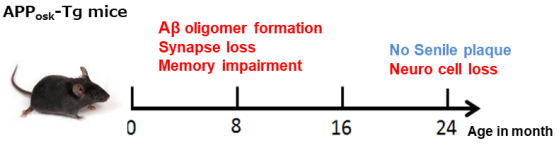
1, Aβ forms soluble aggregate (Aβ oligomer) and insoluble aggregate (Aβ fibril) by auto-aggregation.
2, Aβ aggregate induces hyperphosphorylation and auto-aggregation of TAU protein, and abnormal aggregated TAU (=NFT) is formed.
3, NFT induces neuronal cell death.
4, Cognitive impairment is developed as brain atrophy progresses, leads to AD.



In the early amyloid hypothesis, it was believed that neuronal cell death induced by Aβ fibril (insoluble aggregate in senile plaque) causes cognitive decline. However, since Aβ level necessary for neuronal cell death is too high compared with the actual condition in vivo, and also severity of AD patients does not correlate with the amount of Aβ fibril in the brain, the existence of toxic substance other than Aβ fibril was suggested. Consequently, "Oligomer hypothesis", a theory that Aβ oligomer leading synaptic dysfunction in physiological concentration might be important for AD development, was advocated. However, since both Aβ oligomer and Aβ fibril exist in the brain of AD patients, it was difficult to determine which factor contributes to AD pathogenesis.

The research group of Dr. Takami Tomiyama and Dr. Hiroshi Mori (Osaka City University) identified the novel gene mutation by screening familial AD patients in 2008[(1)]. The mutation in amyloid precursor protein ("APP") which causes one amino acid deletion was named "Osaka mutant" (APP_{osk}; E693Δ). Aβ peptides produced from APP_{osk} , lacking glutamate at position 22 (E22Δ), shows unique property of enhanced oligomerization but no fibrilization. Senile plaque was not detected in the brain of AD patient with Osaka mutant. Consequently, it was proved for the first time that AD is developed by Aβ oligomer alone.

APP_{osk} tg-mice express APP_{osk} mutant protein in the brain[(2)]. APP_{osk} tg-mice develop the accumulation of Aβ oligomer accompanied by aging, however, there is no senile plaque even at 24 months of age. On the other hand, various AD symptoms including synapse loss, hyperphosphorylation of TAU protein, activation of gliocyte, and nerve cell death, are observed in APP_{osk} tg-mice. From these results, APP_{osk} tg-mice are considered to be the mice model supporting "oligomer hypothesis", and also helpful for the research of AD pathogenesis by Aβ oligomer, therapeutic development, and drug discovery [(3)].



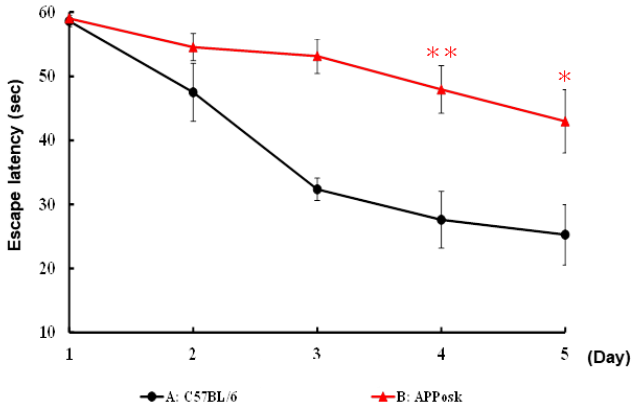
◆Memory test of APP_{osk} tg-mice and Aβ oligomer immunostaining

<Water maze test>

（data acquired by TRANS GENIC）

Animals used： C57BL/6 mice（n = 6）、10 months of age

APP_{OSK} mice （n = 8）、 9～10 months of age

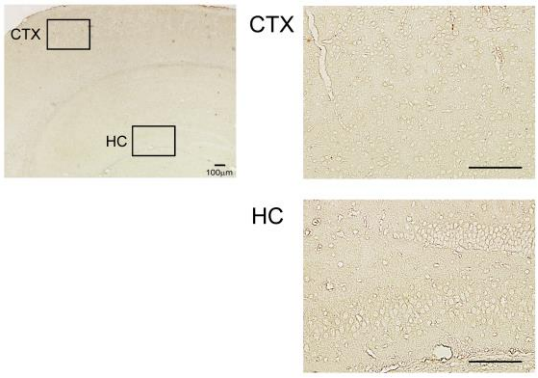


Data shown: average±standard error

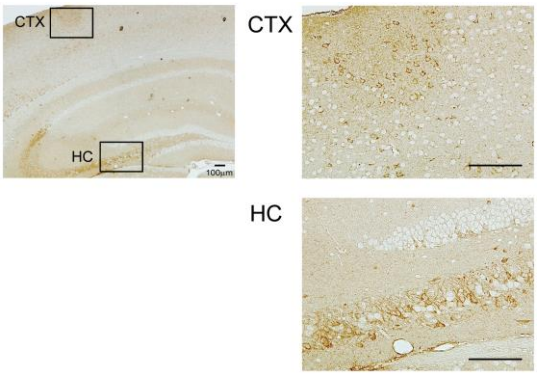
* p<0.0, ** p<0.01, T-test shows significant difference from C57BL/6 mice.

<β-amyloid oligomer staining（immunostaining using NU-1 antibody）>

Non-Transgenic mice



APP_{osk}-Transgenic mice



<Reference>

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A new amyloid β variant favoring oligomerization in Alzheimer's-type dementia. Ann. Neurol. 63, 377-387 (2008).

(2) Tomiyama, T., Matsuyama, S., Iso, H., Umeda, T., Takuma, H., Ohnishi, K., Ishibashi, K., Teraoka, R., Sakama, N., Yamashita, T., Nishitsuji, K., Ito, K., Shimada, H., Lambert, M.P., Klein, W.L. and Mori, H. A mouse model of amyloid β oligomers: Their contribution to synaptic alteration, abnormal Tau phosphorylation, glial activation, and neuronal loss *in vivo*. J. Neurosci. 30, 4845-4856 (2010).

(3) Umeda T., Ono K., Sakai A., Yamashita M., Mizuguchi M, Klein W.L., Yamada M., Mori H., Tomiyama T.
Rifampicin is a candidate preventive medicine against amyloid β and tau oligomers. Brain 139, 1568-1586 (2016).

<Patent>

WO/2006/038729 “Mutated Amyloid Protein”

Mice model of dementia

There are various kinds of dementia such as AD, and frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) is known as an autosomal dominant disease. The signs and symptoms such as motion-impairment and cognitive dysfunction usually become noticeable in a person's forties to sixties, and after a certain period, affected individuals may develop serious dementia. It is known that FTDP-17 is caused by the abnormality of TAU protein. Abnormal accumulation of TAU protein is considered to be directly associated with neural death, and the cause of AD. Some genetic mutations on TAU gene are already reported, including N279K mutation. This is the point mutation causing amino-acid replacement.

SJLB mice express TAU protein with N279K mutation in the brain. SJLB mice exhibit the impairment of spatial ability and risk avoiding ability, therefore, are considered to be useful as the mice model of cognitive impairment to investigate the treatment method and therapeutic agents targeting TAU protein.

<Reference>

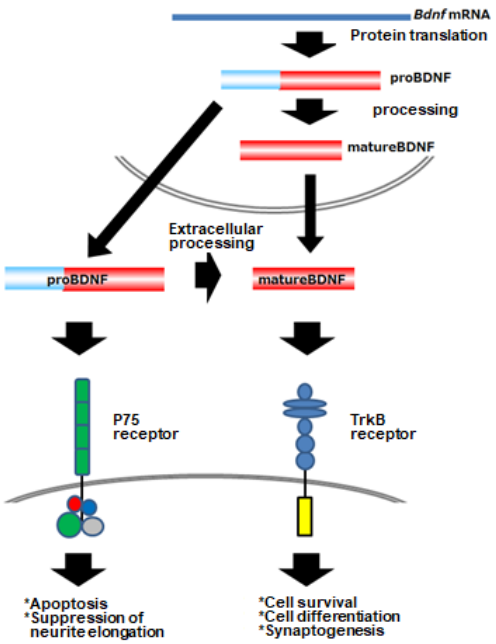
- Taniguchi, T., Doe, N., Matsuyama, S., Kitamura, Y., Mori, H., Saito, N. and Tanaka, C. Transgenic mice expressing mutant (N279K) human tau show mutation dependent cognitive deficits without neurofibllary tangle formation. FEBS letters 579, 5704-5712 (2005).

＜Patent＞

■JP2011043428A　“Inspection method of Parkinsonism using non-human animal model”

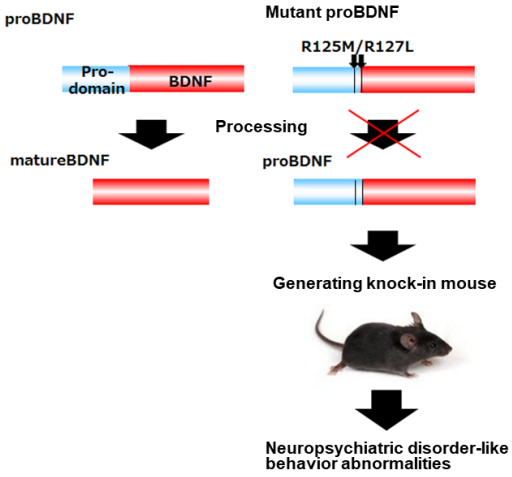
Mice model of neuropsychiatric disorder

Brain-derived neurotrophic factor (BDNF) is one of the neurotropic factors, and has received the attention to be a substance relevant to various neuropsychiatric disorders such as depression in recent years. It is shown that BDNF is important for the formation, development, and retention of neural network, synaptic plasticity, as well as memory formation and learning. Mature BDNF is generated by the cleavage of proBDNF, precursor of BDNF. Mature BDNF binds to tyrosine kinase receptor (TrkB), and induces cell survival, cell differentiation, and synaptogenesis. On the other hand, proBDNF binds to p75NTR receptor, and induces apoptosis and suppression of neurite elongation.



Dr. Masami Kojima (the National Institute of Advanced Industrial Science and Technology) considered that impaired processing of proBDNF and deficient secretion might be involved in neuropsychiatric

disorder, and generated the knock-in mice in which mutation to suppress proBDNF processing is introduced. Consequently, this type of mice exhibited various symptoms: distinctive behavior abnormalities such as titubation and falling while walking, hyperactivity in an open field, and prolonged immobility time in the tail suspension test. From these facts, mutant BDNF gene-introduced knock-in mice can be used as mice model of neuropsychiatric disorder including dementia, and may contribute to the therapeutic development and drug discovery.



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- Koshimizu, H., Hazama, S., Hara, T., Ogura, A. and Kojima, M. Distinct signaling pathways of precursor BDNF and mature BDNF in cultured cerebellar granule neurons. Neurosci. Letters 473, 229-232 (2010).
- Koshimizu, H., Kiyosue, K., Hara, T., Hazama, S., Suzuki, S., Uegaki, K., Nagappan, G., Zaitsev, E., Hirokawa, T., Tatsu, T., Ogura, A., Lu, B. and Kojima, M. Multiple functions of precursor BDNF to CNS neurons: negative regulation of neurite growth, spine formation and cell survival. Mol. Brain 2009 Aug 13;2:27

<Patent>

■JP5414012B2 **“**Mutant BDNF gene-introduced knock-in mice”

Contact for inquiries and additional information：
TRANS GENIC INC.
Yutaka Funabashi, Director
Telephone +81-(0)3-6693-9571