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A new Avenue to Alzheimer's disease based on transgenic mice

Alzheimer's Disease

Alzheimer's disease (AD) is predominantly a disease of the elderly; it's prevalence is increasing almost exponentially as the population grows older. AD robs its sufferers of their memory, their personality, their ability to care for themselves and social context. The slowly developing personal devastation is also reflected in the financial burden to countries worldwide. US Medicare spending on Alzheimer's disease in 2008, for example, was $94 billion, one third of its total budget. There is currently no marketed drug that slows or stops the progression of this disease. Available drugs treat the symptoms, but only transiently.

Pharmaceutical companies have spent large efforts in trying to come up with a disease-modifying drug for AD. However, the last decade has seen a series of failures in late-stage Phase 3 trials. The reasons for these failures are manifold and may include choosing the wrong underlying molecular target.

While much of the research efforts has focused on amyloid, a novel approach has targeted the other key pathology, i.e. neurofibrillary tangles that Dr. Alois Alzheimer described already in 1906. However, it was not until the 1960's that the team of Sir Martin Roth pointed to a correlation between the load of tangles and the degree of clinical dementia. When isolated and purified by Claude Wischik in the 1980s, the nature and composition of these tangles was determined. They are made up of tau, a protein that is actually essential for the shape of neurons and for transport of «cargo» along the axons that connect nerve nets the brain. Any abnormal accumulation of tau in neurons is closely linked with both the clinical signs of dementia and neuronal decay.

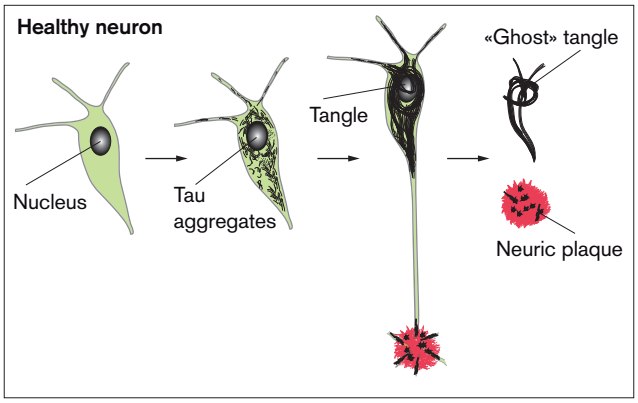

Fig. 1: Schematic representation of the time course of neuronal tau pathology

This spread of tau pathology follows a characteristic anatomical pattern over time that was first discovered and described by the German neuropathologists, Heiko and Eva Braak in 1991 and is since commonly referred to as Braak staging. Tau pathology begins in the hippocampus and entorhinal cortex, a region important in memory and learning in both mice and humans. As the disease progresses, tangles spread over to the temporal, parietal and frontal cortices. This characteristic spread of the tau aggregation pathology seen post-mortem closely matches the regional pattern of brain deficits which can be demonstrated by functional brain scans during life, measuring either reduced blood flow or reduced glucose utilisation (by SPECT or PET). Such metabolic deficits match the clinical progression of AD which can be traced by various neuropsychological test batteries.

In 1991, John Hardy's team in London reported that a genetic mutation led to AD in a patient having a family history of the disease. The mutation affected a protein that gives rise to a fragment of insoluble -amyloid which accumulates outside neurons in the brain in «plaques», the other conspicuous pathology described already by Alois Alzheimer. This

«Any abnormal accumulation of tau in neurons is closely linked with both neuronal decay and the clinical signs of dementia»

gave rise to what was termed the amyloid cascade hypothesis in which the process leading to amyloid accumulation in plaques became central to the process of development of the disorder. This hypothesis has yet to be confirmed in a successful clinical trial.

Braak and Braak showed that -amyloid pathology appears to be a general feature of the aging brain that does not follow any clear pattern of progression or spread. It has since been confirmed that there is minimal if any systematic relationship between -amyloid neuropathology and progression of clinical cognitive decline. Interestingly, «neuritic» plaques in AD also contain nerve terminals that show abundantly the same fibrils as those found in tangles.

Tau Protein Aggregation

To develop drugs that have potential to arrest the process of tau protein aggregation that leads to tangles in AD brains, it is necessary to have a model in which drugs can be tested. Wischik and coworkers developed such models to show that a class of compounds inhibits the aggregation of tau in a test tube, in an isolated cell and then in a mouse model. In both the cellular and mouse models, the process of tau aggregation was initiated by a small seed upon which further tau protein is captured, leading to a stable, insoluble complex of aggregated tau. It is still not understood what starts this process in the human brain, but it seems likely that there may be various events that could trigger this process. It might be abnormal amyloid in some cases or it may be the accumulation of abnormal proteins or of «aging pigment». Once initiated, the capacity of tau protein pathology to propagate itself at the expense of normal tau has been confirmed in several studies recently. Aggregated tau fibrils can be taken up by cells containing full-length tau and passed on to neighbouring cells to seed aggregation. Similarly, the transmission and spread of tau pathology can be induced in mouse models.

Once the initial seeding has occurred, the tau aggregation cascade is self-propagating, leading to two detrimental outcomes. First, it converts the neuron's normal functional tau into an aggregated form found in tangles. Normal tau protein is required to stabilise axonal microtubules. The microtubular network contributes to the shape of the neuron together with its lengthy axon that is required to connect distant parts of the brain. More importantly, the tau aggregates are directly neurotoxic and lead to neuronal death.

Tau Protein Aggregation Inhibitors

Methylthioninium chloride (MT), commonly referred to as methylene blue, was the first tau aggregation inhibitor reported. The MT concentrations effective in the disruption of tangles in vitro and in cellular models are relevant to those safely achieved in humans. This lead to the question of whether MT could prove safe and efficacious in AD.

Therefore, two mice models were developed demonstrating that firstly, tau aggregation creates cognitive and motor learning deficits; and second, tau pathology develops within neurons and in a staged process similar to the human neuropathology.

These mice models confirmed that MT: (a) crosses the blood-brain-barrier; (b) effects a change of clinical phenotype (cognitive capability and motor learning); (c) decreases oligomeric tau load in regions important for memory.

Generation of transgenic mice

Fertilised oocytes were recovered from four week old female mice. The vector, containing tau coding sequence (tau cDNA) under control of a neuronal Thy-1 promoter, was microinjected into the male pronuclei of the fertilised oocytes. After microinjection, the oocytes were implanted into the oviduct of 8-10 week old pseudopregnant female NMRI mice. Transgenic mice were identified by genotyping using PCR (polymerase chain reaction) to identify presence of human-specific, inserted tau DNA. The copy number of inserts was determined by Southern blot. Expression of tau mRNA by Northern blot and tau protein were then determined by immunoblots and immunohistochemical analysis of brain tissue.

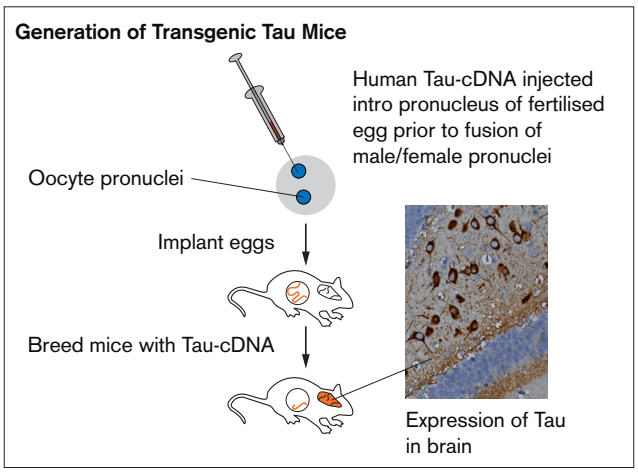

Fig. 2: Generation of Transgenic Tau Mice

In the first transgenic model, «Line 1» mice express a fragment of tau that forms the central unit within the individual filaments of the Alzheimer tangle. The protein is engineered in such a way that it becomes targeted to a membrane location whereby it has the opportunity to initiate a tau seed that can capture further tau protein. These mice recapitulate the essential phenomena of Braak staging. For mice less than 12 months of age, the aggregated tau pathology is located predominantly in the entorhinal cortex and hippocampus. As mice age, the pathology spreads into other cortical brain regions.

These Line 1 mice develop a learning impairment after about 7 months of age, as shown in a water maze task. Line 1 mice require about twice as many trials to reach a given learning ability but oral administration of MT reverses this learning deficit while oligomeric tau load is decreased. MT also shows positive effects in a second mouse model («Line 66»). In this mouse, human tau has been engineered with mutations that accelerate tau aggregation. These mutations are in fact found in another type of dementia – frontotemporal dementia – where the primary symptoms are often behavioural rather than memory changes. Whereas the Line 1 mouse exhibits very modest tau pathology, the Line 66 mouse exhibits severe tau pathology. Tau tangles in Line 66 mice are filamentous and observed in hippocampus (shown below – dark brown), entorhinal and other cortical areas.

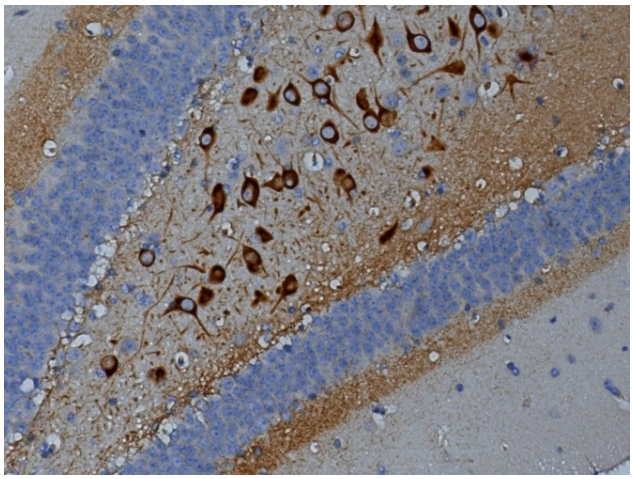

Fig. 3: Immunohistochemical staining of tangles in entorhinal neurons

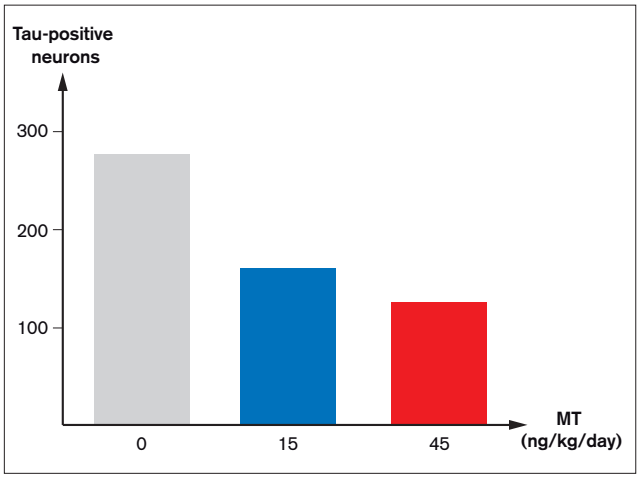

Fig. 4: Dose-dependent reduction of neuronal tau load in mice after MT administration

Line 66 mice show a severe abnormality of motor learning. Oral administration of MT can reverse this deficit while the tau load be decreased.

Thus MT can reverse symptoms and pathology in two transgenic tau mouse models: a cognitive phenotype model with predominant tau oligomers (Line 1) and a frontotemporal-like motor phenotype with abundant filamentous tau (Line 66). The effects

«The effects of Methylthioninium in tau transgenic mice were decisive to encourage clinical research»

of MT in tau transgenic mice were thus decisive to encourage clinical research.

Translational Research in Alzheimer's Disease
The tau aggregation inhibition concept was confirmed by results from a well-controlled clinical trial in 321 AD patients. MT reduced the rate of clinical cognitive decline significantly by 84% over 50 weeks when measured by the ADAS-cog scale, a validated and accepted instrument to capture cognitive change in AD. The co-primary global clinical endpoint and also the results from a SPECT sub-study were significantly positive. These results may suggest that MT has the potential to slow deterioration in AD; the administration of MT might thus be particularly useful at early stages of the disease. An improved form of MT is to be tested in Phase 3 studies in the near future. Confirmation of these initial clinical and neuroimaging findings is eagerly anticipated to see if the rationale for tau-targeted therapy can offer new hope in the treatment of AD.

It would be ideal if we could understand the complicated mechanisms of a body without stressful animal experiment. Unfortunately that is not yet possible today, although researchers have for a long time conducted countless experiments with cells and tissues and, in the age of system biology, are also increasing our knowledge by means of computer simulation. But the dilemma will remain for a long time to come: basic research without experiments in animals would mean abandoning any medical progress. Mausblick aims to explain why and therefore reports on medical success stories that were only possible thanks to animal experiments.

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