



## ***Molecular Mechanisms of Neurodegeneration***

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### **Introduction**

The 2007 premier scientific meeting on Molecular Mechanisms of Neurodegeneration, organized by Abcam and Rudy Tanzi from Massachusetts General Hospital and Harvard Medical School, attracted 120 participants from around the world, including many academic scientists as well as industrial scientists and a few clinicians. This well-prepared event saw presentations by 23 invited speakers and also featured 40 poster presentations, all of which were well-attended and allowed for extensive discussion of each topic. In addition, group dinners and free time on the beach encouraged networking opportunities.

After a warm welcome and opening remarks by Bill Campbell and Dr Tanzi, the meeting commenced with a keynote talk followed by presentations in four broad areas: genetics of neurodegenerative diseases, APP and Abeta, mechanisms of neurodegeneration, and therapies. This report covers recent advances in molecular mechanisms of neurodegenerative diseases as well as novel therapeutic strategies in the field. The program focused heavily on Alzheimer's disease (AD) but also included a talk on Friedreich's Ataxia (FRDA) and several posters on other neurodegenerative diseases. In general, mechanisms discussed included amyloid precursor protein (APP)-binding proteins, Abeta dimers as a toxic species, the role of kinases in tau phosphorylation and Abeta generation, neuroinflammation associated with vascular amyloid, protein degradation, and herpes simplex virus type I (HSV-1). Therapeutic strategies featured included metal-protein attenuating compounds (MPAC), Abeta immunotherapy, a P75<sup>NTR</sup> ligand small molecule and gamma-secretase inhibitory molecules. In addition, the use of human recombinant erythropoietin (rEPO) for treatment of FRDA was presented.

### **Synaptotoxicity of soluble Abeta dimers**

Dennis Selkoe (Brigham & Women's Hospital and Harvard Medical School) presented the keynote address in which he described the isolation of soluble Abeta dimers from late-onset human AD brain and the ability of such dimers to impair hippocampal function and memory in rats. Human Abeta dimers reduced long-term potentiation (LTP) and dendritic spine density in rat hippocampal slices. In addition, intracerebral ventricular infusion of Abeta dimers in rats impaired memory in a passive avoidance behavioral task indicating a direct effect of this human Abeta species on brain function. Lastly, cores isolated from human AD brain were found to contain mostly Abeta dimers but were biologically inactive, suggesting a possibly protective sequestering of Abeta dimers in cores.

### **The role of AICD in EGFR signaling and apoptosis**

In vitro and in vivo data to support novel functions for the APP metabolite APP Intracellular Domain (AICD) generated by presenilin-associated gamma-secretase cleavage of APP were discussed by Huaxi Xu from the Burnham Institute. The absence of one or two presenilin 1 (PS1) alleles in mice results in increased skin tumor formation. In fibroblasts, PS-associated gamma-secretase deficiency resulted in enhanced biosynthesis of EGFR. AICD, but not nicastrin ICD, another substrate of gamma-secretase cleavage, reduced EGFR levels, and AICD was found to bind the EGFR promoter and regulate its expression. In addition, a novel APP/AICD-binding protein, appoptosin, discovered using a yeast two-hybrid system, was described. Appoptosin induced caspase-dependent apoptosis and cytochrome C release in vitro. In the absence of APP, cell death was reduced in cells co-transfected with the C-terminus of appoptosin. It was concluded that these data provide two new functions for AICD in cell signaling and apoptosis and may lead to new therapeutic targets.

### **Neuroinflammation and behavioral deficits in a mouse model of CAA**

SUNY Stony Brook's Bill Van Nostrand described studies in his transgenic mouse model (TgSwDI) bearing low levels of 3 human familial APP mutations (Swedish, Dutch and Iowa), two of which result in severe cerebral amyloid angiopathy (CAA), using a Thy 1 promoter. These mice develop abundant vascular amyloid in subiculum and thalamus as well as diffuse Abeta deposition in cortex, reduced cerebral blood flow, gliosis and impaired memory and learning in the Barnes maze test. Pro-inflammatory cytokines and complement proteins were also elevated. TgSwDI mice aged 11 months were treated with the anti-inflammatory drug minocycline daily for 4 weeks. Abeta deposition and biochemical levels were

unaltered. Instead, microglial activation and pro-inflammatory cytokines (IL-6, IL-1beta, TNF) were suppressed and performance improved in the Barnes maze, suggesting that reducing neuroinflammation associated with CAA may have beneficial effects on memory and learning. In collaboration, with Carol Colton of Duke University, Dr Van Nostrand's animals were crossbred with nitric oxide synthase-2 (NOS2) knockout mice resulting in neuron loss, tau phosphorylation, and worsening of the behavioral impairment.

### **A potential protective role for Cdk5 in AD**

Cdk5 is a proline-directed serine/threonine kinase that has been implicated in tau phosphorylation and neurodegeneration in AD, leading to the idea of the development of Cdk5 inhibitors as a therapy for AD. Karl Herrup (Rutgers University) presented an opposing view in which he described Cdk5 as having a potentially beneficial role in blocking cell cycle initiation. Cell cycle proteins and DNA replication have been observed in regions of neuronal loss in AD brain. Knocking out Cdk5 in mice resulted in poor neuronal migration and survival in brain. Cdk5 protected against Abeta-induced toxicity in primary neuron cell cultures, and knocking out the kinase in these cultures resulted in increased nestin levels, increased mitosis and the presence of cyclin D-positive cells, indicating initiation of cell death. Thus, Cdk5 appears to hold cells in the cell cycle thereby preventing cell death.

### **Mechanisms for the development of tau pathology**

Karen Duff from Columbia University presented evidence for the opposing roles of Cdk5 and GSK3beta in tau phosphorylation and pathology. P25, cleaved from P35, regulates Cdk5 activity, and when overexpressed in young transgenic (tg) mice (generated by Pfizer Pharmaceuticals) leads to increased Cdk5 activity. This correlates with increased phosphorylated tau at certain epitopes such as S235, but decreased phospho-tau levels overall. This counterintuitive result was explained by the observation that p25 tg mice had reduced activity of GSK3b. Inhibiting Cdk5 resulted in increased GSK3beta activity and more phospho-tau protein. Thus, it appears that Cdk5 negatively regulates GSK3beta. One way that Cdk5 mediated GSK3 activity was through activation of ErbB (neuregulin signaling) which activates PI3k/Akt leading to phosphorylation of GSK3 at the inhibitory site. It would appear that of the two kinases, Cdk5 is not the major tau kinase in vivo. This has implications for therapeutic development as inhibition of Cdk5 might enhance the activity of GSK3beta, resulting in elevation of pathological forms of tau and exacerbation of neurodegenerative disease. A study on the effect of p25-mediated activation of Cdk5 on Abeta production showed that Cdk5, rather than GSK3, was the dominant kinase mediating the generation of Abeta. However, inhibition of GSK3 or Cdk5 can significantly impact Abeta production. In the p25 mice, and also in inducible p25 transfected cells, p25/Cdk5 regulates BACE levels. In young mice (but possibly not older mice), p25/cdk5 can regulate BACE through transcriptional control. One transcription factor known to be a substrate for Cdk5 that was implicated in the response was STAT3. To address whether tau hyperphosphorylation had a functional impact in terms of microtubule stability in vivo, Dr Duff described a system whereby hyperphosphorylation is induced by anesthesia-mediated hypothermia. In normal mice, tau binding to microtubules was not negatively impacted by hyperphosphorylation, whereas in mice with tauopathy, hyperphosphorylation reduced tau/microtubule binding. These data suggest that hyperphosphorylation per se is of little detriment except in situations where the brain is compromised. Lastly, Dr Duff described a new transgenic rat model bearing human mutant APP and PS1, developed by Cephalon. These rats develop diffuse Abeta plaques around 9 months of age and extensive compact plaques within 19 months of age. Aged 7 months, they had deficits in LTP, and aged 13 months, cognitive impairment in the Morris water maze.

### **IGF-1 signaling in aging and protein aggregation**

Protein misfolding and aggregation are thought to play roles in neurodegenerative diseases that occur late in life, but how does the aging process influence protein aggregation and visa versa? Andy Dillin (Salk Institut) presented data in worm and mouse models showing that insulin-like growth factor (IGF)-1 signaling is important in determining longevity via its capacity to regulate transcription factors, DAF16 and HSF-1, which, in turn, influence protein aggregation. The speaker generated transgenic worms that overexpress intracellular Abeta1-42 peptide, and display muscle paralysis in mid-life. If IGF-1 signaling was suppressed in these animals, longevity was increased and Abeta toxicity was delayed. In additional experiments, Dr Dillon found that extracts from DAF-16 tg worms converted low-molecular weight Abeta aggregates to higher-molecular weight aggregates, perhaps a less toxic species, in vitro. Extracts from HSF-1 tg worms caused disaggregation of Abeta aggregates to monomers that may be proteolyzed more readily by proteosomes relative to aggregated Abeta species. Atomic force microscopy studies indicated that a trimeric Abeta species may be responsible for the toxicity observed in these models. These studies were then confirmed in mice by crossbreeding APP/PS1 tg mice with IGF-1R mice bearing one or two IGF-1R alleles. The crossed mice with only one IGF-1R allele performed better on the Morris water maze spatial memory test and had higher levels of Abeta monomers compared to those mice with two IGF-1R alleles, even though total Abeta levels were not altered. These results suggest that temporal inactivation of insulin/IGF-1 signaling with aging may promote life extension.

## **LTP enhanced in neprilysin-deficient mice**

The neutral endopeptidase neprilysin (NEP) is a zinc metalloproteinase responsible, in part, for Abeta degradation, but having metabolic effects on other proteins as well. Doris Albrecht (Charite-Universitätsmedizin Berlin) presented data demonstrating that while wild-type mice showed an age-dependent reduction in LTP (induced by a high frequency stimulus) by 9 month of age, the reduction in LTP was delayed to about 24 months of age in NEP-deficient mice. Recordings were made from the lateral nucleus in the amygdala and the CA1 region of hippocampus in horizontal brain slices. Abeta levels were elevated in NEP-deficient mice compared to wild-type animals at both ages. Extracellular Abeta deposition was not observed in any of the NEP-deficient mice, even at 22 months of age. Neuropeptide candidates important for memory and learning, such as glucagon-like peptide and galanin, are substrates for NEP and are under investigation as possible therapeutic targets.

## **HSV-1 infection in brain as a possible cause of AD**

Ruth Itzhaki from the University of Manchester presented in vitro and in vivo data in mice in support of her earlier findings of an association between HSV-1 in brain and AD, including the presence of latent HSV-1 in the brains of many aged people and the elevated risk of AD in those with HSV-1 and one or two apolipoprotein (Apo) E4 alleles. PCR analysis was used to detect HSV-1 DNA in human brain. High levels were found in AD and normal aged controls compared to young controls. In situ, HSV-1 DNA was found in neurons and glia in sections in human AD brain. HSV-1 DNA was observed in more than 90% of plaques in human AD brain. HSV-1 antibodies were detected CSF in 52% of AD and 69% of age-matched elderly controls while HSV-1 antibodies were not present in infant CSF. To further delineate the effects of HSV-1 infection on AD pathogenesis, Dr Itzhaki performed in vitro studies in a variety of cell lines, including neuroblastoma cells, and found increased Abeta production and tau phosphorylation, especially at serine214. Components of the enzymes required to generate Abeta (BACE and nicastrin) were elevated in the infected cells but APP levels were unchanged. In addition, HSV-1 increased the levels of PKA and GSK3 $\beta$  kinases, the latter of which regulates tau phosphorylation and possibly Ab production. HSV-1 infection in wild-type mice resulted in Abeta deposition into plaques as demonstrated by immunohistochemistry. From these results, Dr Itzhaki proposes the use of antiviral agents and possibly, vaccination against HSV-1 as a therapeutic strategy to prevent AD.

## **ACAT inhibitors help reduce Abeta deposition and neurodegeneration in an AD mouse model**

Acyl-coenzyme A:cholesterol acyltransferase (ACAT) produces lipid deposits inside macrophages leading to foam cell formation in atherosclerosis. In addition, cholesterol homeostasis has been implicated in regulation of Abeta generation. ACAT inhibitors prevent the conversion of cholesterol and fatty acids into cholesteryl-esters and are under development for atherosclerosis. Dora Kovacs (Massachusetts General Hospital and Harvard Medical School) presented data showing that two different ACAT inhibitors reduced Abeta generation in vitro and in vivo. The first compound, CP-113818 (Pfizer) reduced Abeta levels in APP tg mouse brain and significantly inhibited plaque deposition when administered to young mice. Next, a second ACAT inhibitor, CI-1011 (avasimibe; Pfizer), was tested in APP tg mice based on its longer half-life and because it is in phase III trials for cardiovascular disease. Two doses of CI-1011 (4.8 mg/kg/day vs 14.4 mg/kg/day) were administered to 4-month old APP tg mice, prior to the onset of AD pathogenesis. In addition, the higher dose was given to 14 mo-old APP tg mice, well after the onset of pathogenesis, for 2 months. Hippocampal and cortical plaque burdens were markedly reduced in the prevention trial in the younger mice. A dose-dependent reduction in plasma Abeta40 and Abeta42 was also observed. Cerebral plaque burden was significantly reduced in the treatment trial in the older mice, although no change was observed in fibrillar, thioflavin S-positive plaques, suggesting that CI-1011 was most effective at removing diffuse Abeta deposits. Synaptophysin levels, typically reduced in old APP tg mice, were restored with CI-1011 treatment. In vitro studies using ACAT siRNA also reduced APP processing by reducing Abeta and APP CTF levels. Further studies are underway to determine the mechanism of action of ACAT inhibitors in reducing APP processing and Abeta generation.

## **P75NTR ligands to prevent Abeta-induced neurodegeneration**

P75NTR is a pan-neurotrophin receptor expressed on neurons in AD-affected brain regions. P75NTR may contribute to Abeta peptide-induced deleterious signaling and degeneration in neuronal cells in vitro to signal cell death. However, P75NTR is also involved in pro-survival signaling via PI3K/Akt and NF $\kappa$ B pathways. Juliet Knowles presented data generated in the laboratory of Frank Longo from Stanford University using novel small-molecule P75NTR ligands to block Abeta-induced neuronal death and neuritic dystrophy in primary neurons and hippocampal brain slices. P75NTR ligands were able to block death induced by Abeta oligomers in primary hippocampal, septal and cortical neurons in vitro. Importantly, p75NTR ligands were unable to block Abeta toxicity in neurons deficient for P75NTR, indicating that the protective effect was specific for P75NTR. Oral dosing with a lead p75NTR ligand in APP tg mice from 4 to 7 months of age resulted in improvement in performance in novel object recognition to the level of age-matched non-tg mice. Further preclinical studies are underway to prepare for human clinical trials.

## Targeting metal interactions to lower Abeta levels

Colin Masters (University of Melbourne) presented data on preclinical studies to lower Abeta levels using metal-protein attenuating compounds (MPACs) *in vitro* and *in vivo* in PSAPP tg mice. Abeta has previously been shown by Drs Ashley Bush and Rudy Tanzi to possess two copper-zinc binding sites which promote oligomerization and the reduction of copper, resulting in the generation of hydrogen peroxide and hydroxyl radicals. Abeta bound to copper, in the presence of hydrogen peroxide, has also previously been demonstrated to generate dityrosine cross-linked Abeta oligomers, eg dimers and trimers. Dr Masters presented his new data showing that Abeta must bind cell membranes to be toxic, although only L-handed Abeta1-42 but not D-handed Abeta1-42 stereo-isomers are toxic *in vitro*. In primary neuronal cultures, both D- and L-Abeta1-42 bound to the neuronal cell surface. Annexin V inhibited this binding and partially inhibited the toxicity of L-handed Abeta1-42. In an effort to reduce the formation of Abeta dimers, MPACs (clioquinol and PBT-2 from Prana Biotechnology, LTD) were used to block binding of Abeta to the cell membrane. However, in clinical trials conducted five years ago, clioquinol had only showed significant improvement in cognition in mild AD patients compared to placebo controls. This drug was discontinued by Prana Biotechnology, and replaced by its new 8-hydroxy-quinoline derivative, PBT-2, referred to as the 'son of clioquinol' by Dr Masters. The speaker went on to present new data in which both clioquinol and PBT-2 were able to reverse Abeta-induced inhibition of LTP in rodent hippocampal brain slices. When PBT-2 was tested in PSAPP tg mice, Abeta levels were reduced in the interstitial fluid and returned to baseline following drug washout. In addition, total tau was reduced while phospho-tau was increased slightly. Synaptophysin levels and dendritic spine density in CA1 neurons in hippocampus were significantly increased, and the PBT-2-treated mice showed improved spatial learning and memory in the Morris water maze test. Results of Prana's phase II trial of PBT-2 in around 80 subjects will be released in early 2008. Lastly, Dr Masters described a large study in which 11C-PIB (Pittsburg Imaging Compound) was used to image approximately 200 subjects in Melbourne, Australia. PIB imaging in AD and Diffuse Lewy Body Disease (DLB) patients was similar but both showed more labeling than in mild cognitive impairment (MCI) patients (intermediate) and FrontoTemporal Dementia (FTD) patients (little binding). In general, Abeta load by PIB imaging correlated with memory loss.

## Novel Abeta vaccine and mechanisms of Abeta clearance by immunotherapy in AD mice

Abeta immunotherapy data using two different AD-like mouse models were discussed by David Cribbs from the University of California Irvine. In the first study, a second-generation DNA vaccine was administered by gene gun to immunize 3xTg-AD mice, bearing human mutant APP, PS1 and tau transgenes. The DNA vaccine, pMDC-3Abeta1-11-PADRE, encoded a Th2-biased molecular adjuvant (macrophage-derived chemokine; MDC), three copies of Abeta1-11 peptide, and a pan-specific human T-cell epitope, PADRE, to avoid an Abeta-specific T-cell response while generating enough T-cell help to mount a robust humoral immune response. Transgenic mice were treated from 3 to 18 months of age and generated high titers (1:300,000) consisting of mostly IgG1 antibodies, indicating a predominant Th2 effect. Abeta deposition and Abeta oligomers were significantly reduced in the Abeta-vaccinated mice compared to non-vaccinated mice. Tau levels (total tau using HT7 antibody) were unchanged, and microhemorrhages were not observed. Further studies are underway to determine treatment effects of this vaccine in older mice after the onset of AD pathogenesis. In a second study, Dr Cribbs vaccinated TgSwDI mice with his Abeta1-11-PADRE peptide vaccine using Th1-biased Quil A as an adjuvant. Although the mice generated anti-Abeta antibodies, cerebral Abeta levels were unchanged. Anti-Abeta antibodies were isolated from the blood of immunized mice and administered directly into the brains of TgSwDI mice. In this case, the antibodies were effective in lowering cerebral Abeta presumably because they could directly interact with Abeta without having to rely on Abeta transport across the blood-brain barrier.

## Active Abeta vaccines stabilize or improve cognition and lower plaque burden in primates

APP and Abeta sequences are homologous between non-human primates (NHP) and humans, and both develop cerebral Abeta plaques with aging. In a study presented by Cindy Lemere (Brigham & Women's Hospital and Harvard Medical School), aged Caribbean vervets (mean age approximately 20.5 years) were given seven intramuscular injections of either aged synthetic Abeta1-42 peptide (n = 7) or dendrimeric Abeta1-15 (dAbeta1-15; 16 copies of Abeta1-15 on a lysine tree; n = 7) with adjuvant QS-21, or adjuvant alone (n = 6) over a period of 9 months. Prior to the study, four animals per group were trained to perform novel object recognition (NOR), and delayed non-match-to-sample (DNMTS) using the monkey version of the Cambridge Neuropsychological Test Automated Battery (CANTAB) using a computer touch screen system with a food pellet reward. Vervets immunized with Abeta1-42 generated moderate-to-high anti-Abeta titers whereas titers were lower in dAbeta1-15 immunized animals using Abeta1-40 coated ELISA plates; one animal did not produce anti-Abeta antibodies (ie, a non-responder). Antibodies from all seven Abeta1-42 immunized vervets and four of seven dAbeta1-15 immunized animals bound human and APP tg mouse cerebral Abeta plaques. Cortical plaque burden was reduced in both vaccine groups, but no significant changes were observed in CSF Abeta levels due to high variability. Microhemorrhage was not observed in brain tissues from Abeta-vaccinated vervets. Cognitive testing was performed in a blinded fashion just prior to the start of immunization and again 9 months later, just before the animals were euthanized. Cognitive performance

declined in vehicle control animals whereas immunization with Abeta1-42 stabilized cognition. Surprisingly, immunization with dAbeta1-15 significantly improved cognitive performance from pre- to post-immunization compared to the vehicle control group. Although anti-Abeta titer levels did not correlate with cognitive improvement, there was a significant correlation between cognitive improvement and Abeta plaque burden in both vaccine groups compared to vehicle controls when the non-responder was removed from the analysis. This study was supported by funding from ELAN and Wyeth.

### **Small-molecule gamma-secretase modulators selectively lower Abeta42 levels in vitro and in vivo**

Abeta42 has been implicated in the pathogenesis of AD, therefore, selectively reducing Abeta42 levels has become a therapeutic target. Gamma-secretase has a number of known substrates making inhibition of one substrate without affecting the others tricky. Steve Wagner (TorreyPines Therapeutics) presented evidence that selective modulation of gamma-secretase using small molecules (Series 555) shifted the C-terminal cleavage of Abeta from the generation of Abeta ending at residue 42 (and to a lesser degree at residue 40) to Abeta ending at residues 38 and 37, both thought to be less amyloidogenic than Abeta42. Compound NGX-83232 reduced Abeta42 generation in neuroblastoma cells in a dose-dependent fashion. A subgroup of Series 555 small molecules also inhibited Abeta42 generation but did not affect Notch processing (another gamma-secretase substrate) or notch intracellular domain (NICD) formation. These compounds also lowered Abeta40 but required higher doses. In APP Tg mouse primary neuronal cultures (Tg2576) and cell-free assays, these small molecules lowered Abeta42 and 40 and increased generation of Abeta37 and Abeta38 without changing overall Abeta levels. These results were confirmed in vivo in Tg2576 mice treated orally on a daily basis with the small molecules for 5 days. In addition, 3 days of oral treatment with 25- to 50-mg/kg daily doses reduced Abeta42 plasma levels. No toxicity or safety issues were observed. Currently, the compounds are being incorporated into rodent chow for further long-term testing for prevention and/or treatment in APP tg mice.

### **Promising early results using rhuEPO for FRDA**

FRDA is caused by a GAA-trinucleotide expansion in the frataxin gene, encoded on Chromosome 9q13. As a result, frataxin expression, a mitochondrial protein, is reduced, leading to reduced cell energy and enhanced free radical production. FRDA is a slow, progressive neurodegenerative disorder with a typical onset in patients under 20 years of age. Patients are usually wheelchair-bound within 15 years of onset and life expectancy is reduced. Erythropoietin is a cytokine that regulates erythropoiesis and hemoglobin synthesis. Although it is routinely used as anti-anemia therapy in dialysis and cancer patients, rhuEPO is also under investigation for neuroprotective and cardioprotective effects. Barbara Scheiber-Mojdehkar (Medical University of Vienna) presented recent clinical trial data using rhuEPO in FRDA patients. First, 12 patients were treated with 5000 IU rhuEPO three times per week for 8 weeks, resulting in increased frataxin in eight patients. Following a washout period, all patients were then treated with 2000 IU three times per week for 6 months. Significant reductions were observed in the Friedreich's Ataxia Rating Scale, especially in areas of locomotion and speech, indicating improvement in the rhuEPO-treated FRDA patients. Further studies are in progress.

### **Summary**

Presentations at this meeting covered a very broad range of mechanisms and therapeutic strategies for the prevention and/or treatment of AD, but also included FRDA. As new mechanisms for neurodegeneration are uncovered, new therapeutic targets are revealed. Promising therapies for AD presented here included ACAT inhibitors, small-molecule neurotrophin receptor ligands, metal-interacting compounds, Abeta immunotherapy, and small-molecule gamma-secretase modulation of Abeta42. While most of the data reported were derived from preclinical investigations, several clinical studies are underway or in the pipeline. Lastly, rhuEPO looks promising for the treatment of FRDA by increasing frataxin levels.

The website for this meeting can be found at

<http://www.abcam.com/index.html?pageconfig=resource&rid=10794&source=pagetrap>