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Keystone Symposium 2010 Highlights

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Alzheimer Research Forum

Knight Vision – SIRT1 Aids ADAM10, Slays Amyloid-β

Unseasonably warm days gave way to some notable nights at this year's Keystone Symposium, Alzheimer's Disease Beyond Aβ, held 10–15 January at Copper Mountain, Colorado. One evening offering was a short talk from Gizem Donmez, a postdoctoral fellow in Leonard Guarente's laboratory at MIT. Donmez reported that SIRT1, the histone deacetylase linked to longevity, might protect against AD by boosting ADAM10 (aka α-secretase) and promoting non-amyloidogenic processing of amyloid-β protein precursor (AβPP). If true, then you might want to eat more carrots because the effect seems to rely on SIRT1 playing vassal to the retinoic acid receptor.

SIRT1 is activated by caloric restriction, which protects against brain atrophy in primates. SIRT1 itself also protects against neurodegeneration in mouse models of AD [1], and previous work from Giulio Pasinetti's laboratory at Mount Sinai School of Medicine, New York, suggested that activation of α-secretase may be responsible [2]. Pasinetti and colleagues attributed the increase in α-secretase to SIRT1 inhibition of the Rho kinase ROCK1, previously linked to suppression of the non-amyloidogenic secretase). But Donmez's work suggests that there is more to the tale.

To explore the relationship between SIRT1 and AD, Donmez and colleagues made mice with either the SIRT1 gene knocked out or overexpressed. For knockouts, Donmez used the cre/lox system driven by a nestin promoter, limiting SIRT1 loss to neurons. For overexpression, she knocked the SIRT1 gene into the β actin locus, getting a mild, twofold overexpression. Donmez tested the effects of the SIRT1 mice on Aβ pathology by crossing them with AβPP/PS1 transgenic animals (AβPPSwe/PS1ΔE9).

Donmez reported that the AβPP/PS1/SIRT1 knockouts die earlier than control AβPP/PS1 animals, and that the knockouts have increased amyloid plaques and gliosis. The increased pathology in these mice was accompanied by a reduction in α-secretase activity. In contrast, AβPP/PS1 mice overexpressing SIRT1 had reduced levels of Aβ_{42} compared to controls and increased ADAM10 and ADAM10 mRNA. Levels of Notch intracellular domain, which is produced following α-secretase processing of the transmembrane receptor, were also increased when SIRT1 was overexpressed but not when it was knocked out. The results support the theory that SIRT1 can boost expression of the secretase.

Donmez jousted with the ADAM10 promoter using chromatin immunoprecipitation assays to determine exactly how SIRT1 might exert its influence. She reported that the deacetylase attaches to the promoter very close to a binding site for the retinoic acid receptor (RAR)/retinoid X receptor (RXR) heterodimer. Activation of the ADAM10 gene depended on SIRT1 deacetylase activity (an inactive mutant has no effect) and also the presence of retinoic acid. The evidence suggests that SIRT1 deacetylates RAR leading to increased expression of ADAM10, presumably by allowing RAR to bind more tightly to the promoter. In support of this, Donmez found that RARβ is deacetylated in the presence of SIRT1 and that RARβ acetylation is increased in SIRT1 knockout cells. Coming back full circle, she showed that she was able to reverse the reduced production of Aβ in SIRT1-overexpressing cells by knocking down ADAM10 transcripts with RNA interference.

Donmez concluded that SIRT1 activators might be worth pursuing as potential therapeutics for AD. Resveratrol, a SIRT1 activator found in miniscule quantities in red wine, is widely promoted in the popular

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press as an elixir of life. It has received serious attention from the scientific community as well, since it has been shown to mimic some of the effects of caloric restriction) though other research counters that blocking SIRT1 might actually improve cognition). Resveratrol, however, does not cross the blood-brain barrier very efficiently. Amongst all of this, vitamin A, which is metabolized to retinoic acid, might be worth a closer look, too. Recent findings suggest that all-trans retinoic acid can protect AβPP/PS double transgenic mice against Aβ pathology, reducing levels of the peptide without affecting AβPP [3], while acitretin, a vitamin A analog, was also shown to upregulate ADAM10 [4]. Because acitretin crosses the blood-brain barrier and has been approved for treating psoriasis since 1997, it would appear to be a candidate for exploratory clinical or preclinical studies.

RAT-A-TAT – NEW MODEL COMES A KNOCKIN'

Bigger, smarter, and more amenable than mice to the imaging techniques that are rapidly becoming indispensable in AD research, rats could be a valuable model for studying AD. The major downside of the sagacious creatures is that they are about five times more expensive to maintain than mice, but then again, maybe you get what you pay for. For all the research into mouse models, many still come up short, failing to recapitulate some of the most basic AD pathologies, such as neurofibrillary tangles and neuronal loss. What if you could have it all in one animal?

That could soon be possible, according to Terrence Town, Cedars-Sinai Medical Center, Los Angeles, California. At the recent Keystone Symposium, Alzheimer's Disease Beyond Aβ, held 10–15 January 2010 at Copper Mountain, Colorado, Town debuted a new rat model at the end of a talk focusing on the role of the innate immune system in AD. The model is the result of collaboration with Robert M. Cohen and Robert Pechnick at the same institution. If the model characteristics Town presented turn out to be true, researchers may be salivating over more than their ratatouille.

The rats express both human AβPP with the Swedish mutation and human PS1 with the exon 9 deletion, a la David Borchelt's AβPP/PS1 mouse [5]. The transgenes are driven by the hamster prion promoter, as in Karen Hsiao Ashe's Tg2576 mice [6]. Town reported that the animals show reduced NeuN staining compared to controls (around 25 percent lower in the hippocampus and a slightly greater loss in the cingulate cortex), suggesting neuronal loss with age. The rats develop plaques that can be detected by FDDNP imaging; importantly, they also develop nearby tangles as seen by immunohistochemistry (using Cp13 and PHF1 antibodies to tau) and ultrastructural electron microscopy. FDDNP imaging discriminates transgenic animals from controls, which opens up the possibility of following pathology longitudinally in individual animals, Town reported. (FDDNP is thought to bind to both plaques and tangles). Caspase 3, a marker of cell death, is also elevated in the AβPP/PS1 rats compared to controls. Levels of the caspase increase with age, and the protein appears in the vicinity of plaques. Tunel staining of 16- and 27-month-old rat brain tissue suggests progressive cell loss in the cingulate and hippocampus, Town said.

Town believes his may be the first AD rat to have a chance of becoming widely used. He noted that he hopes to make it freely available to academic laboratories, though companies may have to deal with some red tape and pay a fee. He suggested these rats better mimic human AD pathology than do similar mouse models because the rat tau proteome is more akin to that of humans. For example, humans express six different tau isoforms that differ by the number (three or four) and type of repeat units and by the extent of inserts in the N-terminal of the protein [7]. Whereas mice express a four-repeat tau exclusively in the brain, a recent study suggests that the rat brain boasts the full complement of six isoforms [8].

The transgenic rats also exhibit gliosis, another hallmark of AD, and interestingly, Town showed confocal microscopy data suggesting that activated microglia (as judged by IBA1 staining) seem to take up both Aβ (seen by ThioS or 4G8 staining) and are filled with tau (Cp13 staining). "This could be a unique form of microgliosis," suggested Town.

This data all seems fairly hot off the press. Town showed no behavioral results, but in response to questions, he did say that the animals show a significant decline in hippocampal-based learning and memory that kicks in around 15 months of age when plaque deposition is evident. He concluded by suggesting that these animals may present a better platform for preclinical testing than the current crop of transgenic mice.

Other rat models that express human AβPP, PS1, or both have been produced in the past [9–13]. It is not clear why these models have not been more widely used, but Town told the Alzheimer Research Forum (ARF) that some of them appear to be short-lived, making them less suitable for AD research, while others

lack the extensive pathology. "One of the key features of our AD rat model is that it produces high levels of total Aβ with age (over 100 microgam/wet gram of brain tissue), and it has an almost 1:2 ratio of Aβ1-42:Aβ1-40," Town told ARF via e-mail. "Perhaps other rat models have not attained the requisite levels/type of Aβ in order to precipitate the full amyloid cascade hypothesis," he suggested.

DEATH AND TROPHIN RECEPTORS – NEW INSIGHT, NEW DRUGS?

Death and taxes are reputedly inevitable, though death and receptors may be more interesting to ARF readers. Both were in season at this month's Keystone symposium, "Alzheimer's Disease Beyond Aβ," held 10–15 January 2010 at Copper Mountain, Colorado. Frank Longo, of Stanford University School of Medicine, California, reviewed evidence for the part the neurotrophin receptor p75 plays in Aβ toxicity, arguing that small-molecule p75 ligands may protect against AD.

This neurotrophin receptor has been linked to AD for some time. For example, as Longo noted at Copper Mountain, p75, like its close relative DR6, is expressed in brain areas most vulnerable in AD, including the entorhinal cortex, hippocampus, and basal forebrain, and expression is elevated in patients with the disease. The receptor mediates Aβ-induced cell death according to data from Elizabeth Coulson's group at the University of Queensland, Brisbane, Australia [14], and recently Longo and colleagues reported that p75 mediates Aβ-induced neuritic dystrophy, one of the key pathological findings in AD [15]. However, other reports suggest p75 can also be protective in AD [16].

Much of the early work Longo described stemmed from the use of p75 knockout (KO) mice. Rather than having the full gene knocked out, these animals are missing the third exon and hence the majority of the receptor. Primary neurons from these knockouts survive in culture and resist Aβ_{42}, showing less dystrophy when exposed to the peptide than do wild-type neurons. Neuritic dystrophy is milder in offspring from AβPP transgenic mice (with Swedish and London mutations) crossed with p75 KO mice. Those offspring also have fewer plaques than the parent human AβPP transgenic strain.

After summarizing some of his laboratory's recent findings, Longo outlined a strategy to develop small-molecule p75 ligands to treat AD. Drug development stages of the work are being done at Pharmatrophix, a startup company Longo co-founded. The company received initial support from the Alzheimer's Drug Discovery Foundation and has since entered a partnership with Elan.

Longo and colleagues, including Stephen Massa at UCSF, have developed small-molecule ligands that bind to p75, block Aβ-induced neurodegeneration, and prevent some of the downstream cascades associated with Aβ toxicity, such as activation of Cdk5, GSK3β, JNK, tau phosphorylation, and inhibition of Akt [17]. Ligands also inhibit the ability of Aβ to block CREB activation. Some of the molecules are active in the picomolar range, Longo said, adding that he has begun testing them in both hippocampal slices and *in vivo* in mouse models of AD.

In cooperation with Mike Shelanski's laboratory at Columbia University, New York, a first set of studies found that the compounds can protect against Aβ-induced loss of dendritic spines in hippocampal slices. Together with Ottavio Arrancio, also at Columbia, the researchers found that the p75 ligands rescue Aβ-induced LTP deficits in hippocampal slices. Several of these compounds get into the brain, Longo said. On going *in vivo* studies are establishing whether the compounds affect neuritic dystrophy and spine loss and if they can reverse behavioral deficits.

ORIGINS AND ACTIONS OF GLIAL CELLS IN AD

There's more to the brain than neurons. Astrocytes and microglia play crucial roles in the development and maintenance of a healthy brain, and both have been studied for their potential protective and deleterious roles in AD. The field grapples with two fundamental questions: What do these cells do, and where do they come from? At Alzheimer's Disease Beyond Aβ, this year's Keystone Symposium held January 10–15 at Copper Mountain, Colorado, presenters tried to answer both. The general impression is that researchers are finally getting some traction in understanding the role of these cells and whether or not peripheral phagocytic monocytes can infiltrate the brain and influence pathological cascades.

One cell type that has been implicated in neurodegeneration is the astrocyte. Less involved in immune responses than microglia, astrocytes lend important trophic support to neurons and protect them from excitotoxicity). But they may also be involved in glial

activation, and one of the proteins they release, the calcium-binding protein S100B, appears to be upregulated in AD [18]. S100B also surfaces around amyloid plaques in AβPP transgenic mice [19]. To probe the role of the protein in AD, researchers led by Terrence Town, Cedars Sinai Medical Center, Los Angeles, and Takashi Mori at Saitama University, Kawagoe, Japan, crossed Tg2576 AβPP transgenic mice with animals that overexpress human S100B. At Copper Mountain, Town reported that at 15 and 19 months of age, the double-transgenic animals have deposited more and larger Aβ plaques in the cingulate cortex, hippocampus, and entorhinal cortex compared to Tg2576 controls. Blood vessels in the double- transgenic animals also contain numerous Aβ deposits, and the mice have higher levels of soluble Aβ and of C-99, and sAβPPβ fragments of AβPP generated by β-secretase (BACE) cleavage. The researchers confirmed elevated BACE activity when they measured it directly.

In addition to more amyloidogenic processing of AβPP, microgliosis (judged by Iba1 staining) and astrogliosis (judged by GFAP staining) emerged in the double transgenic mice by 19 months of age. But at 9 months, before the emergence of any Aβ pathology, proinflammatory cytokines, including tumor necrosis factor α, interleukin 1b (IL-1b), IL-6 and even mouse S100B were up. This work just appeared in the February issue of Glia [20]. The timing of events, with inflammatory signals going off before Aβ begins to accumulate, suggests that brain inflammatory processes are not simply a consequence of plaques but may even drive cerebral β-amyloidosis, suggested Town.

Town also reviewed some of his recent work on TGF-β signaling, which suggests that blocking this pathway can bias peripheral mononuclear phagocytes toward non-inflammatory responses in AD models. The cells' inflammatory tendencies are can be appeased by shunting TGFβ signaling away from the downstream transcription factors Smad2 and Smad3, and toward Smads 1, 5, and 8). Together, the TGF-β and the S100B data demonstrate a delicate balance when microglia or macrophages come into contact with Aβ, suggested Town. He believes the balance might be struck to enable Aβ clearance without setting off an inflammatory cascade by blocking TGFβ signaling and is currently searching for small-molecule inhibitors that might be suitable for preclinical work. To this end, he has entered into a partnership with Novartis Inc, and with a medicinal chemistry laboratory at Yale University, Town told ARF.

Town's data suggest that dialing down TGF-β signaling in peripheral mononuclear phagocytes may open the door for these cells to enter the brain and scavenge Aβ. The role of peripheral macrophages in the brain remains somewhat controversial, however, and its study has been hampered by technical challenges. For one thing, distinguishing brain-resident microglia from infiltrating circulatory macrophages is quite difficult because, when activated, the former express similar markers to the latter. One technique that has been used to explore the role of myeloid cells in the brain is to ablate myeloid-generating bone marrow by irradiation and then transplant new, traceable cells from another animal. In this way, researchers reported that only circulating monocytes expressing the chemokine receptor CCR2 and high levels of the cell surface marker Ly-6C are able to infiltrate the brain [21]. But questions remain as to whether circulating monocytes enter the brain because the blood brain barrier gets damaged by irradiation, as some researchers suspect, and whether those infiltrating cells have any impact on AD pathology.

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