

FOUNDATION HIGHLIGHTS 2018

Biomedical Research and Findings:

Several hallmarks strongly associated with Alzheimer's disease (AD) have been discovered over the years and a protein called APP (for amyloid precursor protein) takes center stage in the formation of what is called amyloid plaques. It is still relatively unclear what the biological function of APP is, even though a number of hypothesis are available. However, it is well known that APP, in the context of the disease, can be cleaved by two other proteins (or enzymes) called beta-secretase and gamma-secretase. The consequence of these two cleavages is the release of a toxic component or peptide (a fragment of a protein) called A β peptide. The self-aggregation of A β peptides leads to the formation of amyloid plaques. A large part of the work carried out by the Fisher scientists is related to various aspects of APP biology. For one of the projects, in relation to neuronal vulnerability, the second major hallmark of Alzheimer's disease will be also investigated.

Other important aspects of the work involve the identification of the molecular mechanisms underlying vulnerability and resistance of neurons. Neurons that are vulnerable, disappear early on during the disease process, while resistant neurons are very similar to the vulnerable cells in appearance, but are more resistant to the disease state. It is not understood why some neurons are more vulnerable than others but if vulnerable cells can be protected pharmacologically, it may potentially hold the key to reverse the disease progression.

Neurons are crucial for all cognitive functions, but other cell types found in the brain are important too. The non-neuronal cells in the brain are often referred to as accessory cells because their main function is to support neurons. A specific type of these accessory cells is called microglia. Microglia is attracting more and more attention because while microglia has an important role in maintaining the brain free of debris, its sur-activation might damage the neurons and become problematic, for example, by chewing away synapses at the surface of the neurons.

Finally, the Fisher scientists recently initiated a novel line of research using a unique type of cells derived from Alzheimer's patients which can be grown in culture to study the importance of various genes and pathways. This type of study aims at identifying factors that could explain the disease state, and designed to validate vulnerability candidate genes identified as described previously.

A brief summary of the most recent findings is presented below.

1) Screening chemical derivatives of Gleevec and mechanisms of action

Fisher scientists have previously demonstrated that the cancer drug called Gleevec, that was known to target protein kinases, has the capacity to lower beta-amyloid. The original version of Gleevec was not extremely potent and was only poorly brain permeable. In order to optimize these two key aspects, different pharmacological programs were put into place. One program was aimed at developing hundreds of true Gleevec analogs. A second project called reverse Gleevec was designed to drastically alter the molecular structure of Gleevec by reversing two parts of the molecule. The third program called DV was centered on making analogs of related compounds belonging to a different class.

Gleevec analogs generated earlier, and described in details in prior progress reports, were tested through mechanistic characterizations and long-term *in vivo* studies. Two compounds were selected, out of approximately 200, that have been extensively tested. These compounds were shown to reduce A β production in cellular assays. *In vivo* efficacy studies have shown that these two selected compounds are clearly accumulating in the brain, significantly more than Gleevec. Fisher scientists also showed promising effects on amyloid plaques for one compound when administered to mice in drinking water for over 3 months. It

was also confirmed that new Gleevec analogs remain functionally similar to the parent compound.

Similar studies with several 'reverse Gleevec' analogs were initiated and are at various level of accomplishment. *In vivo* studies were initiated and will be completed soon.

The compounds called 'DV analogs' are derived from a slightly different approach. Similar to Gleevec, another class of compounds, a kinase inactive analog of a potent dual inhibitor with a different mechanism targeting Abl/Src kinases, has been found to modulate A β production in cellular assays. The Fisher scientists have focused on the analog DV2-103 which strongly reduces A β 40 but does not alter A β 42 level in an *in-vitro* cellular assay. At the contrary, the kinase inactive analog DV2-103 reduces both A β 40 and A β 42 levels. Because it presents added advantage of accumulating significantly in the mouse brain, the 80 analogs of DV2-103 generated previously have now been evaluated in cells. Compounds that strongly reduce A β productions and that are greater than DV2-103 have been identified.

Two manuscripts are being prepared and will be submitted for publication shortly.

2) GSAP-dependent trafficking is essential for A β (Abeta) peptide accumulation

A few years ago, we identified a protein based on its capacity to bind Gleevec in the context of Abeta peptide modulation. We determined that this uncharacterized protein at the time, most likely had a role of gamma-secretase activating protein (GSAP). The mechanistic details underlying gamma-secretase activation, remain obscure due to practical limitation because of the nature of the protein GSAP. Meanwhile, work has also progress on studying another role of GSAP, a role on protein trafficking and especially on APP trafficking. Fisher scientists investigated the role of GSAP on an early trafficking step and showed that this trafficking route may be relevant for APP maturation and therefore for Alzheimer's disease. Our latest studies, using a complex set of imaging technologies coupled to biochemical experiments, are now completed and clearly demonstrate that the GSAP regulates APP intracellular trafficking. Using the state-of-the-art TIRF microscopy, the Fisher scientists found that GSAP knockdown (less GSAP is made in the cells) lowers the total number and the membrane dwell time of surface APP vesicles, indicating a role for GSAP in the membrane retention of APP vesicles. These data therefore define a new role of GSAP on amyloidogenic processing mechanism that involves crosstalk between APP trafficking machinery and A β production. A manuscript is in its final stage and will be submitted for publication soon.

3) Understanding the vulnerability of some specific neurons to disappear early on in the disease

The concept of selective neuronal vulnerability in the context of Alzheimer's disease has been attracting more and more attention over the last few years. This concept of vulnerability implicates that specific types of nerve cells (neurons) will be more susceptible to a pathological process and will be affected and disappear sooner than others and thus be called resistant neurons. The regions of higher vulnerability have been relatively well mapped in humans. A brain region called the entorhinal cortex (EC) is the region of the brain that is the most vulnerable to degeneration (the neurons from this region disappear first), where cell death happens very early on, but it is not known why. This region is extremely important as it communicates directly with the region key for learning and memory called hippocampus. Understanding why some cells are vulnerable and others are resistant to the disease process will certainly help to decipher the underlying causes of the disease and help design novel therapeutic strategies. To pursue the goal of understanding selective neuronal vulnerability in AD, Fisher scientists are using a unique set of tools and technologies that they have developed over the years. The tools developed by the scientists over the years allow them to separate specific neurons from complex mixtures. So far, they have discovered dozens of new genes that are linked to the vulnerability of EC neurons. Among these, Fisher scientists described the

discovery of ADV1 (for AD vulnerability 1), a gene which is mutated in very rare families that have a high incidence of AD, and seems to regulate how excitable an EC neuron, ADV2 and ADV3, can be, that regulate the levels of many proteins involved in the vulnerability of neurons. Finally, ADV4, a « splice factor » that clips out of the Tau protein (the main constituent of the AD-defining neurofibrillary tangles) a fragment that can increase its propensity to aggregate, and which seems to be most active in EC neurons. These four genes, and others that Fisher scientists can now connect to vulnerability, are all candidate genes that could serve as therapeutic targets for the treatment of AD-related neurodegeneration. Further characterization aimed at demonstrating that these genes are indeed involved in AD development are currently in progress. This very ambitious and unique work has been published in 2018 in BioRxiv. A manuscript corresponding to the part of the work made available at BioRxiv will also be submitted for publication in a peer-reviewed journal.

The drug screening platforms, that currently use neurons, are using 'generic' neurons, which do not have the same molecular content as the neurons important in AD, and potentially do not activate the same disease mechanisms when they form AD-related pathology. This represents a major obstacle in developing therapeutic treatments against AD neurodegeneration. Fisher scientists thus decided to take advantage of their study of the most AD-vulnerable neurons (EC neurons), in order to learn how to recreate them from human induced pluripotent stem cells (iPSCs). Namely, they are using a combination of molecular profiles of EC neurons at different stages of their development, and a large compendium of human publicly available databases, in order to predict the proteins that can drive differentiation of stem cells to an EC neuron identity. They are working on establishing and validating a list of candidate genes, and introduce these candidate genes into stem cells. The main goal is to determine if the modified stem cells are acquiring EC molecular and functional characteristics, if they are becoming EC neurons. Once the goal reached, this system will represent an extremely powerful tool to study AD and to screen drug-like compounds in a search of therapeutic drugs.

4) Regulatory role of microglia cells in the context of Alzheimer's disease

Microglia cells represent a sub-group of cells belonging to the larger family of cells called glial cells. These cells function, in the central nervous system, as macrophages or scavengers, that are activated upon insult and then physically directed toward the location where the insult took place. These cells are mobile and can be activated very quickly in response to various cellular signals associated with insults. Following an insult, microglia cells are activated, presenting an increased chemo-sensitivity (they can sense chemical compounds produce locally) and start moving toward the insult zone that is releasing microglia chemo-attractants. Once at destination, the microglia cells will engulf and digest anything that is dysfunctional, misshaped or dead such as dead cells and neuronal debris, synapses in some cases and also amyloid plaques as we have shown previously in another study and using the iDISCO method. Microglia contributes to AD pathogenesis and seems to have various roles, but the mechanisms by which microglia become dysfunctional in AD remain obscure. The Fisher scientists studied how microglial Presenilin 1 (the main active component of gamma-secretase enzyme responsible for one of APP cleavages) specifically modulates microglial function in AD. They have shown that they can modulate AD-like hallmarks by manipulating Presenilin 1, specifically in microglia. They have also shown, using multiple state-of-the-art molecular techniques, that Presenilin 1 plays a crucial role during microglial development which consequently modulates synaptic transmission. All together, these results demonstrate that Presenilin 1 links microglial function to AD. This study has been submitted for publication and is currently being revised.

5) An APP cleavage product called C99, more than Abeta peptide, co-localizes in vulnerable neurons of Alzheimer's patients

As mentioned earlier, the accumulation of the toxic beta-amyloid peptide is believed to cause Alzheimer's disease. However, it has long been puzzling to the Alzheimer research community why beta-amyloid distribution in the brains of Alzheimer patients does not always co-localize well with cells undergoing neurodegeneration, and with neurons that are in the process of dying. Understandably, this discrepancy has been one of the main objections to the concept that beta-amyloid causes Alzheimer's disease. The Fisher scientists have tackle this apparent inconsistency over the years and propose a possible explanation in a new study recently published in BioRxiv. In this new study, the Fisher scientists provide evidence that C99, a cleavage product of APP and the immediate precursor of beta-amyloid, accumulates selectively in vulnerable cells, but not in resistant cells. Furthermore, our data suggest that C99, rather than beta-amyloid, is responsible for neuronal toxicity and provide a new framework for understanding the etiology of Alzheimer's disease. This study will soon be submitted for publication in a peer-reviewed journal.