



Alternative Amyloid Hypothesis for Alzheimer's Disease

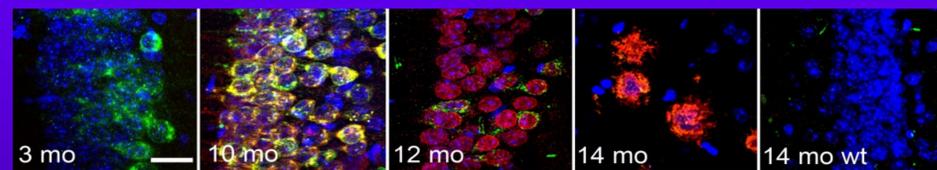
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Background

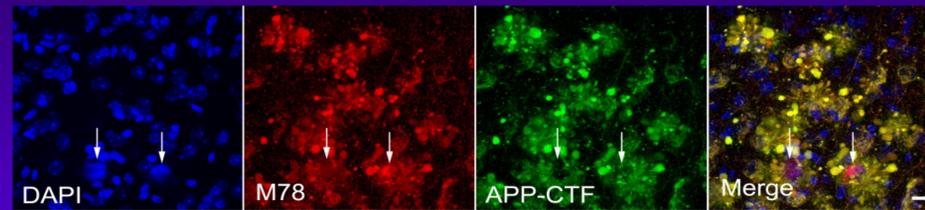
In 2010, Eli Lilly reported the results of human clinical trials of Semagacestat, the first disease modifying drug for Alzheimer's disease. You haven't heard of this breakthrough? The reason is that Semagacestat actually made the patients that received the drug cognitively worse than the patients that received a placebo. Although scientists have many explanations for this unexpected and opposite result, the simplest explanation is that we are thinking about the disease mechanism backwards. Semagacestat was developed based on the prediction of the "Amyloid Hypothesis" which postulates that soluble A β is secreted from neurons and aggregates outside the cell to form the toxic species that cause disease. In science, inverse or mirror image mechanisms can often explain the same biological result. For example, an increase in gene expression can be accomplished either by an increase in the activity of a gene activator or a decrease in activity of a gene repressor. What is the inverse or mirror image of the Amyloid Hypothesis? The amyloid hypothesis states that secretion of soluble A β from neurons is the key event in AD and the inverse would be that the intraneuronal retention of insoluble A β is pathological. There is evidence in transgenic mouse models and human brain that the aggregation and accumulation of insoluble A β begins inside neurons prior to the formation of plaques and that plaques initiate from the death and degeneration of these neurons that have accumulated aggregated and insoluble A β and longer A β containing fragments of the amyloid precursor protein, APP.

1. Aggregation begins inside neurons



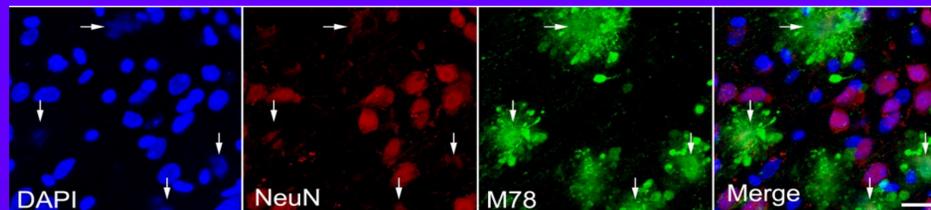
In 3-month-old 3XTg-AD mice, increased levels of APP are observed that stain with an A β specific antibody 6E10 (green). This is normal, unaggregated APP/A β because it is not stained by the aggregation specific antibody M78 (red). At 10 months, the elevated APP/A β in neurons stains with both 6E10 and M78 (yellow), indicating that it is aggregated. At 12 months, the aggregation-specific staining is observed primarily in the nucleus of neurons (red), surrounded by 6E10 staining (green). At 14 months, intraneuronal staining is no longer observed, and instead plaques are found that stain with M78 (red). No antibody staining is observed in wild type (wt) mice. The blue color is DAPI, which stains the DNA in chromatin. These results indicate that aggregation begins inside neurons before plaques are observed.

2. Degenerating neurons with intracellular amyloid aggregates become neuritic plaques.

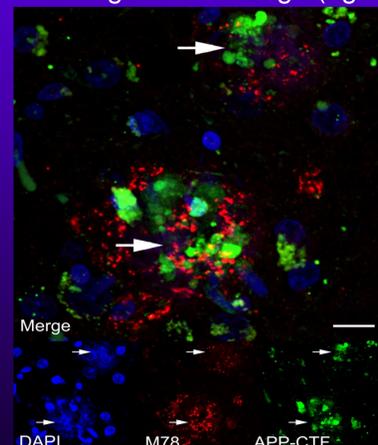


Between 12 and 14 months in 3XTg-AD mice, neuritic plaques, indicated by arrows, are observed that stain with 6E10 (green) and M78 (red). The center of neuritic plaques contains diffuse chromatin that is stained with DAPI (arrows, left panel). The center of the neuritic plaque also stains with M78, just like the nuclei of the neurons at 12 months. The neuritic plaque is surrounded by the carboxyl terminal fragment of APP (APP-CTF, green) staining that co-localizes with M78 immunoreactivity (orange) in the merged color image in the right panel. This indicates that the material is aggregated and that the neuritic plaque has the same spatial relationship of chromatin, M78 and APP-CTF immunoreactivity as seen in neurons at 12 months.

3. The chromatin in the center of neuritic plaques comes from neurons.



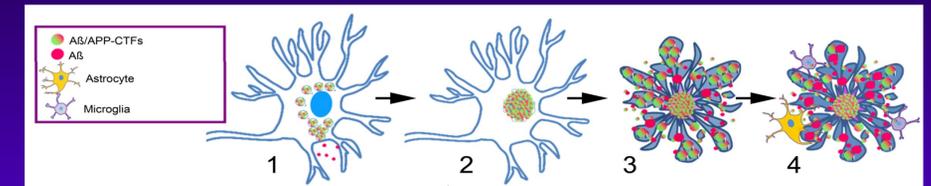
Brain tissue contains several other types of cells besides neurons. To show that the chromatin in the center of neuritic plaques is derived from neurons, we stained 12-month 3XTg-AD sections with NeuN (red), which is a specific marker for neuronal nuclei. The images show that NeuN immunoreactivity is found in the center of each neuritic plaque (arrows). Normal neurons that are not degenerating have nuclei that stain with NeuN in merged color image (right panel).



4. The same type of neuritic plaques are seen in human AD brain.

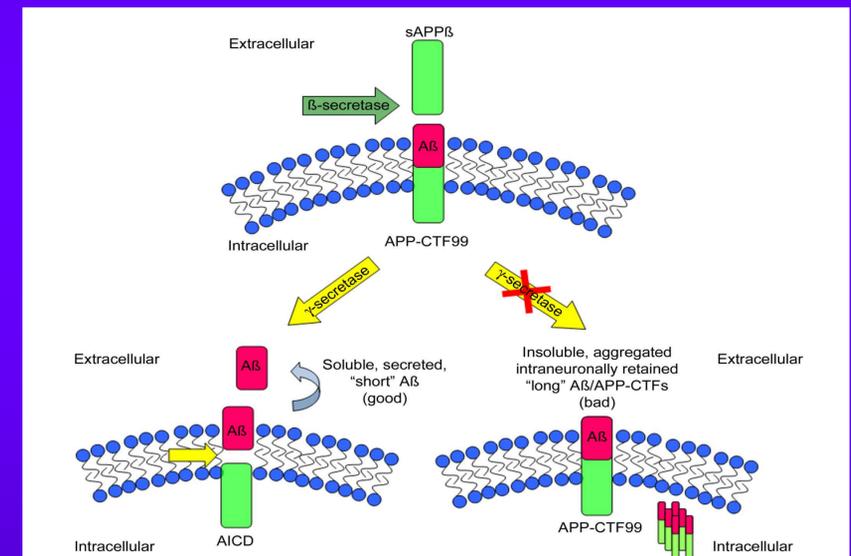
Diffuse chromatin stained with DAPI (blue) and M78 aggregation-specific staining (red) is observed in the center of human neuritic plaques surrounded by APP-CTF staining as observed for 12-month 3XTg-AD mice above.

5. Model for plaque formation and pathogenesis of AD



1. Aggregation begins inside neurons as evidenced by staining with 6E10 and M78 which is specific for amyloid aggregates.
2. Accumulation of M78 positive material continues inside nuclei.
3. The neurons with intracellular amyloid degenerate forming the nidus of a neuritic plaque.
4. Neuritic plaques mature by the action of microglia which internalize and degrade the chromatin and other normal cellular components and leave behind the aggregated A β which is resistant to degradation. The microglia also initiate an inflammatory reaction.

6. Model for an "alternative" amyloid hypothesis.



BACE-mediated cleavage of APP results in the generation of soluble sAPP β and the transmembrane APP-CTF99. The next step in the amyloidogenic pathway consists of the gamma-secretase-mediated cleavage of APP-CTF99. Gamma secretase is a "processive" enzyme that cleaves APP 3 to 4 times to produce soluble A β . This is like eating a piece of spaghetti from one end in several bites. The high processivity (many bites) of wild type gamma-secretase releases soluble, short, "good" A β species in the extracellular space and AICD in the cytosol. If the gamma-secretase processivity is lost (only 1 or two bites) because of FAD-linked PS mutations or gamma-secretase inhibition, the long A β species and APP-CTF99 aggregate intracellularly and accumulate because of their intrinsic resistance to degradation, initiating the pathogenic mechanisms leading to cell death and formation of neuritic plaques. Like FAD mutations in gamma secretase, Semagacestat inhibits the processivity of gamma secretase, which can explain why it made the disease worse. This predicts that gamma secretase modulators that make the enzyme more processive will be effective therapeutic drugs.