Scientific Literacy Assignment

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12 Dec 2021

The article “A novel function for proSAAS as an amyloid anti-aggregant in Alzheimer's disease” explains what, Akina Hoshino et al, have found in their studies. The study shows how protein misfolds and their aggregation can affect the human brain and how it is related to neurodegenerative diseases. It explains that there is a neuroendocrine protein called proSAAS that aids in the abnormal aggregation of misfolded Beta sheets. It was found that both, mice that had Alzheimer’s and humans that had Alzheimer’s had an aggregation of protein in their brain. And the proSAAS helps it deposit there.

## What is protein folding?

A polypeptide must go through protein folding to be functionally active. Sometimes when going through the intermediates they may form a different conformation than the intended result this is called protein misfolding. (Mohammad Krusheed Siddiqi, 2017). Native proteins can also be subjected to misfolding, under stressful conditions, this is because the energy that separates the native protein and the misfolded protein is very small. Since the hydrophobic chain of the misfolded protein is exposed, it will interact with other molecules, which will lead to protein aggregation. Protein aggregation is self-assembly that is caused by the misfolded protein and it has low solubility in water.

The correct folding of a protein is controlled by chaperones and finding a drug that can help block aggregation can help slow down the neurodegenerative disease. (Akina Hoshino, et.al, 2014). Some chaperones are sequence-specific and any change in the polypeptide chain will alter the binding of the chaperon protein to the chain, this will stop the function of the polypeptide chain.

## What is proSAAS and its effect?

ProSASS is a chaperone protein that helps in the circadian rhythm and an anti-aggregant protein. This chaperone is found in both, mice that are 12 months old and humans that are affected by Alzheimer’s disease. The study suggests that this chaperone accumulates the protein in the brain forming a plaque. This plaque is called β-amyloid (Aβ). proSASS is expressed by the brain in high levels but also we can find it in many other cells. Circadian rhythm is a change that follows a 24 hour cycle responding to primarily to light and darkness. This rhythm affects almost all living things.

According to the article, ProSASS has been found in many neurodegenerative diseases such as Alzheimer's disease, Parkinson’s, Pick’s disease. This implies that it may be one of the causes for these diseases to occur. It is a specific inhibitor for prohormone convertase 1/3 (PC1/3), which could cause these problems. It also has been found to have other functions that are essential for our body, Circadian rhythm, food intake, energy balance, etc. This shows that the proSAAS is not only a disease-causing protein and that it needs more study to be conducted.

The method used to study the effect of proSAAS is, the scientists had a tissue sample of a 73-year-old man who had Alzheimer’s disease. It was preserved in formalin, the tissue was sectioned and blocked for an hour, this helps to stain and see the protein and tissue sections. After this process, it was incubated with the anti-proSAAS and anti- $A\beta_{17\text{-}26}$, which were raised in a rabbit and a monoclonal mouse respectively. The anti-proSAAS, which was used in

pancreatic tissues before, was prepared by incubating it and rinsing it by goat anti-rabbit and the anti- $A\beta_{17-26}$ was prepared by incubating and rinsing it with donkey anti-mouse blocking solutions. After the preparations, the slides were viewed and merged. The anatomical localization of the immunoreactivity was then explained according to the Allen Human Brain Atlas and Gray's Anatomy of the Human Body.

To determine if proSAAS directly interact with β-amyloid in the living body of the animal, a co-immunoprecipitation experiment was conducted. Co-immunoprecipitation is the isolation of an antigen using a specific antibody to a sedimentable matrix, this helps with understanding protein-protein interactions. B-amyloid was immunoprecipitated from a 12-month-old mouse then stained with the anti-serum directed against the proSAAS raised in the rabbit. The result showed an immunoreaction band consistent with the proSAAS(1-180).

One of the findings was that proSAAS prevents fibrillation of $A\beta_{1-42}$. It was found that the detergent-insoluble, misfolded proteins like β- sheet fibrils bind to dyes such as ThT. Thioflavin T (ThT) is used to monitor the amyloid fibril formation in vitro. The scientists then tested if proSAAS would block the formation of $A\beta_{1-42}$ fibrils and it was found that the addition of 21k Da mproSAAS (N- terminal), which was found in the mouse, prevented the fibrillation. It was a highly potent inhibitor of fibrillation, in a controlled dose. The $A\beta_{1-42}$ fibrillation was decreased in half by the low molar ratio of 37 to 1, 37 moles of $A\beta_{1-42,}$ and 1 mole of proSAAS. After a while, it was retested and it showed that the length of $A\beta_{1-42}$ was shortened by 75%.

Anti-fibrillation synthetic peptides were used to find the region within the proSAAS that is responsible. It was predicted that the N-terminal domain contained three α-helices. The scientists wanted to see if all the α-helices were required to prevent fibrillation or if one was sufficient; it was then discovered that α-helices II and III were both required and that the protein-coding for these two α-helices might be necessary for proper folding of a protein.

It was also seen that the N-terminally shortened proSAAS constructs showed neuroprotection against $A\beta_{1-42}$ induced cytotoxicity, which is the level at which substances can cause severe damage to a cell. But this only worked for constructs 3 and 4, it did not protect on constructs 5 and 6 even though it was added externally. It was found that internally released proSAAS protects from cytotoxicity by 40% but the effect of the protection on secreted proSAAS is still unknown since it is difficult to study. In general, it was found that both internally released or externally added proSAAS helped in the protection of the cell.

In figure 6a the graph shows how the increased amount of time in incubation also increased cytotoxicity, and figure 6c shows that adding the proSAAS encoding virus (proSAAS LV) increased the amount of proSAAS in the body. Figure 6b slide i shows, a normal functioning cell, slide ii shows, how $A\beta_{1-42}$ fibrillation would affect the cell, slide iii shows adding 21k Da mproSAAS help revive those cells back to normal, slide iv shows, how adding a-lactalbumin did not make a difference on the affected cell. Figure 6d shows that how siRNA added proSAAS decreased cell viability.

## Conclusion

In conclusion, it was hypothesized that proSAAS had other functions than just PC1/3 inhibition. From just the data that was found it was hard to conclude if the localization of proSAAS was functional or non-specific. It was observed that at different stages proSAAS modulated plaque functions, this is because at the early stage proSAAS localization diffused plaque formation, and at a later stage, it was found to be localized at a very dense core pathology.

In the study, proSAAS immunoreactivity was found within amyloid plaques and it was difficult to understand why, but it is hypothesized that it may be due to in certain parts of the brain the concentration of $A\beta_{1-42}$ might be greater than the proSAAS which will make it harder to block the plaque formation. As a result of this, if there is a higher concentration of plaque it will decrease the availability of proSAAS to prevent cytotoxicity and fibrillation which will lead to different types of neurodegenerative diseases. The other hypothesis is that the inappropriate hydrophobic interactions could contribute to fibril formation, and proSAAS can block fibrillation by preventing these interactions between the hydrophobic regions.

In the future, proSAAS inducing treatment would help prevent Alzheimer's disease. This would be achieved by either specific cell therapy or by manufacturing medicine that will increase the production of proSAAS in the body. The other method could be by weakening the negative impact of the proSAAS inducing virus and administering that as a vaccine. In general, this study will help in creating a vaccine or medicine for neurodegeneration disease sooner than later.

# References

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