

Use of yeast-based apoptosis system for exploring treatment options for Alzheimer's disease

One of the most common neurodegenerative disorders affecting the current elderly population is Alzheimer's disease. It is characterized by problems with memory, decline in cognition, increasing dependence for small tasks and, ultimately, death. It usually occurs due to the deposition of amyloid β peptides in specific regions of the brain forming plaques and accumulation of protein tau filaments forming intracellular neurofibrillary tangles. However, there is still a number of pathological elements which are not clear and more research is needed to discover more proteins contributing to the Alzheimer disease pathways (Vos et al., 2011).

Apoptosis is an important regulatory pathway in normal physiological processes and plays significant roles in homeostasis, tissue remodeling, elimination of infection, and DNA damage and repair processes. The proteins involved in apoptotic pathways are conserved from yeast to humans, and this makes yeast an important and reliable model system for studying human apoptotic mechanisms and regulation. It has been shown that expression of mammalian pro-apoptotic proteins, such as those belonging to the bcl-2 protein family, can be lethal in yeast, and this lethality can be balanced by the co-expression of anti-apoptotic proteins. Hence, this experimental system can be exploited for the discovery of those proteins that can prove useful in blocking cell death in several disease pathways, such as in Alzheimer's disease (Weinberger et al., 2003).

In most neurodegenerative disorders, the most common regulatory mechanism that has been recently identified is Programmed Cell Death (PCD) or apoptosis. In the case of Alzheimer's disease, there is an increased expression of pro-apoptotic proteins such as p53, c-Jun and c-Fax, and anti-apoptotic proteins such as Bcl-2 and Bcl-X. All these proteins form an

important part of cell death pathways in a neurodegenerative brain (Jellinger and Stadelmann, 2001).

Yeast, due to its ease of use, reliability and convenience, is a very important model system for the study of various neurodegenerative disorders. It has been used historically for the isolation and functional study of human genes and this has been possible due to conservation of most important pathways between yeast and higher eukaryotes. However, apoptosis is an exclusively multicellular feature which is absent from unicellular organisms like yeast. The introduction of human pro- and anti-apoptosis inhibitors in the yeast system induces human-like pathways and hence, makes it an attractive system for the study of human apoptosis (Greenhalf et al., 1999).

As cell death is an important feature of Alzheimer's disease, yeast is a reliable system for identifying apoptosis inhibitor molecules to prevent widespread cell death pathways in an affected brain. This is possible by using specific yeast strains with plasmids and appropriate selection systems. Yeast strains should be prepared by introducing a chemically synthesized Bax expression cassette under a strongly inducible *GAL10* promoter. A cDNA library should be prepared by using tissue extracts from the human cerebellum and cloned in a yeast-compatible plasmid between appropriate restriction sites. Another plasmid vector belonging to *Escherichia coli* should also be used to allow for easy cloning of PCR-amplified DNA fragments in yeast. A third plasmid should be used to allow for selection in yeast. A fourth plasmid should carry genes that will allow for mammalian expression of the yeast and a fifth plasmid should be introduced carrying the gene for Green Fluorescent Protein (GFP) (Greenhalf et al., 1999).

Yeast cells carrying these plasmids should be grown on suitable media and lysed and the DNA should be extracted. This DNA should be introduced into a competent cell strain and

transformed cells identified by PCR of an appropriate DNA fragment and growth on appropriate selection media. As the Bax expression cassette was introduced under a galactose promoter, cells were tested to see if they were sensitive or resistant to galactose. If there is an expression of any protein that can nullify the effect of Bax, the cells will not be able to grow in the presence of galactose. In such a case, the total DNA should be extracted from the cells and tested to screen for appropriate inhibitors of Bax. These could be potential inhibitor molecules of apoptosis that could be used for the treatment of Alzheimer's disease (Greenhalf et al., 1999).

This system has been widely exploited for the discovery of a number of human proteins involved in various disorders. The uses of yeast as a model system are many and has been extensively used in different fields of research. In the case of Alzheimer's disease, cell death being a key feature in the pathogenesis, the yeast-based apoptosis system is highly useful for the purpose of therapy.

References

- Greenhalf, W., Lee, J., & Chaudhuri, B., 1999. A selection system for human apoptosis inhibitors using yeast. *Yeast*, 15, pp. 1307-1321.
- Jellinger, K. A., & Stadelmann, C., 2001. Problems of cell death in neurodegeneration and Alzheimer's disease. *Journal of Alzheimer's disease*, 3, pp. 31-40.
- Vos, A. D., Anandhakumar, J., Brande, J. V., Verduyckt, M., Franssens, V., Winderickx, J., & Swinnen, E., 2011. Yeast as a model system to study tau biology. *International Journal of Alzheimer's disease*, 2011.
- Weinberger, M., Ramachandran, L., & Burhans, W. C., 2003. Apoptosis in yeasts. *Life*, 55 (8), pp. 467-472.