New application of directed evolution

——exploring the pathogenesis of Alzheimer's disease.

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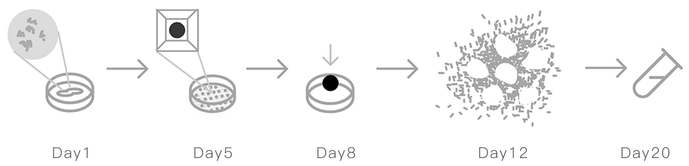
1·Background

Alzheimer's disease is a central nervous system disorder characterized by progressive cognitive dysfunction and memory loss, clinically characterized by generalized dementia manifestations such as memory impairment, aphasia, apraxia, agnosia, impaired visual space skills, executive dysfunction, and personality and behavioral changes. Alzheimer's disease is characterized by two main pathological lesions in the brain, amyloid plaques and neurofibrillary tangles. Among them, neurofibrillary tangles are caused by abnormal overphosphorylation of Tau protein and loss of normal biological function. Recent studies have shown that extracellular vesicles wrapped in the abnormal Tau protein are easily internalized by normal neuronal cells, causing disease damage to metastasize and expand. Key genes affect the exosome release of Tau, which causes the accumulation of Tau aggregates, the formation of NFTs, and the clinical phenotype of AD.

Directed evolution is to simulate the Darwinian evolutionary process in a test tube, through random mutations and recombination, artificially crte a large number of mutations, give selection pressure according to specific needs and purposes, screen out proteins with desired characteristics, and achieve simulated evolution at the molecular level.

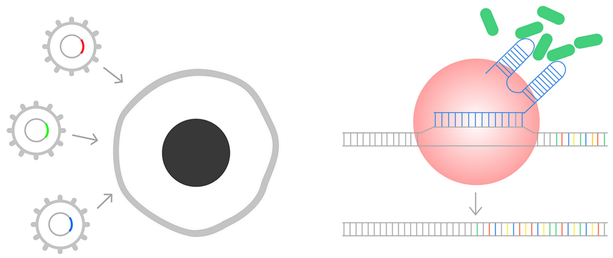
2·Method

1. Generating NPC from iPS cells.



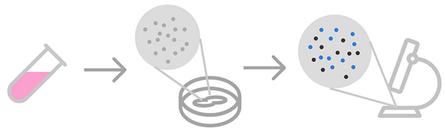
Day0:Plating iPS cells to special plates to generating EBs.
Day5: Replating EBs.
Day8: Neural induction.
Day12: Neural rosette selection and replating.
Day20:Collecting NPCs.

2.Directed evolution.



Three plasmids (enzyme 1, enzyme 2 and sgRNA) were integrated into the cell genome using lentivirus vectors. SgRNA will recruit enzyme 1 and enzyme 2 to specific sites to trigger DNA repair reaction, and errors will occur in the process of sequence self repair to form mutations.

3. Screening cells.



The exosomes containing tau were enriched by physical methods and inoculated into NPC. The content of tau in cells was detected by immunofluorescence, and the cells without abnormal enrichment were screened out to study its essence from the molecular level.

3·Expectation

Directed evolution techniques are currently commonly used to alter protein properties, increase protein activity and expression, and are rarely used to study disease pathology. It is expected that the technology of directed evolution can be applied in more fields.

Reference:

[1] Hess GT, Frésard L, Han K, Lee CH, Li A, Cimprich KA, Montgomery SB, Bassik MC. Directed evolution using dCas9-targeted somatic hypermutation in mammalian cells. Nat Methods. 2016 Dec;13(12):1036-1042. doi: 10.1038/nmeth.4038. Epub 2016 Oct 31. PMID: 27798611; PMCID: PMC5557288.
[2] Ruan Z, Pathak D, Venkatesan Kalavai S, Yoshii-Kitahara A, Muraoka S, Bhatt N, Takamatsu-Yukawa K, Hu J, Wang Y, Hersh S, Ericsson M, Gorantla S, Gendelman HE, Kayed R, Ikezu S, Luebke JI, Ikezu T. Alzheimer's disease brain-derived extracellular vesicles spread tau pathology in interneurons. Brain. 2021 Feb 12;144(1):288-309. doi: 10.1093/brain/awaa376. Erratum in: Brain. 2021 May 7;144(4):e42. PMID: 33246331; PMCID: PMC7880668.