Title
Studies on therapeutics of prion diseases: Establishment of novel screening method for anti-prion compounds and cell therapy model [an abstract of dissertation and a summary of dissertation review]

Author(s)
単智夫

Citation
北海道大学. 博士(獣医学) 甲第12617号

Issue Date
2017-03-23

Doc URL
http://hdl.handle.net/2115/65651

Rights(URL)
http://creativecommons.org/licenses/by-nc-sa/2.1/jp/

Type
theses (doctoral - abstract and summary of review)

Additional Information
There are other files related to this item in HUSCAP. Check the above URL.

File Information
Zhifu_Shan_abstract.pdf (論文内容の要旨)
Studies on therapeutics of prion diseases –Establishment of novel screening method for anti-prion compounds and cell therapy model–
(プリオン病の治療法に関する研究 —抗プリオン薬新規スクリーニング法と細胞治療モデルの確立—)

Immortalized cells that permit prion propagation, such as neuroblastoma cells, have been used for the screening of anti-prion compounds. However, one of the technical limitations is the requirement for proteinase K (PK) treatment to remove cellular isoform of prion protein (PrP<sup>C</sup>) from the cells. It is well known that abnormal isoform of prion protein (PrP<sup>Sc</sup>) comprises PK-sensitive PrP<sup>Sc</sup>-sen (PrP<sup>Sc</sup>-sen) and PK-resistant PrP<sup>Sc</sup>-res (PrP<sup>Sc</sup>-res), and that PrP<sup>Sc</sup>-sen is reported to possess higher infectivity and conversion activity than PrP<sup>Sc</sup>-res. PK treatment digests PrP<sup>Sc</sup>-sen so that the effect of compounds on PrP<sup>Sc</sup> formation may be overlooked. Therefore, in the Chapter I, I established a novel cell-based ELISA in which PrP<sup>Sc</sup> can be directly detected from prion-infected cells using anti-PrP monoclonal antibody (mAb 132) without PK treatment. MAb 132 could detect both PrP<sup>Sc</sup>-sen and PrP<sup>Sc</sup>-res even if all PrP<sup>Sc</sup> molecules were not detected. The analytical dynamic range for PrP<sup>Sc</sup> detection was approximately 1 log. The coefficient of variation and signal-to-background ratio were 7%–11% and 2.5–3.3, respectively, demonstrating the reproducibility of this assay. The addition of a cytotoxicity assay immediately before PrP<sup>Sc</sup> detection did not affect the following PrP<sup>Sc</sup> detection. Thus, all the procedures including cell culture, cytotoxicity assay, and PrP<sup>Sc</sup> detection were completed in the same plate. The simplicity and non-requirement for cell lysis or PK treatment are advantages for the high
throughput screening of anti-prion compounds.

Another direction for the therapeutics of prion disease is the protection of neurodegeneration. The autologous mesenchymal stem cells (MSCs) transplantation has been reported to show tendency of functional recovery or partial improvement in patients of stroke in neurodegenerative disorders such as stroke and spinal cord injury. In prion diseases, it is reported that allogenic transplantation of human MSCs mitigated the disease progression in prion-infected mice. However, autologous MSCs transplantation is required for the practical application for the patients. Thus, in the Chapter II, I evaluated the efficacy of mouse MSCs transplantation into prion-infected mice. Intra-hippocampus transplantation of compact bone-derived MSCs (CB-MSCs) at 120 days post inoculation marginally but significantly prolonged survival of mice infected with the Chandler prion strain. The CB-MSCs transplantation did not influence the accumulation of PrPSc; however, the CB-MSCs transplantation enhanced microglial activation that appeared to be polarized to M2-type activation state. These results suggest that the autologous MSC transplantation is possible treatment for prion diseases and that modification of microglial activation may be a target for therapeutics of neurodegenerative diseases.

In this thesis, I established the novel cell-based ELISA for the screening of anti-prion compounds. Since the mechanism of PrPSc detection differs from other screening methods used thus far, it should have potential for finding new therapeutic compounds. I also showed that the autologous transplantation of MSCs prolonged the survival of mice infected with prions. The results open the possibility of regenerative medicine for the prion disease. I believe that the results described in this thesis will accelerate further researches on the establishment of therapeutics for prion diseases.