Objectives: Recent studies described that the expression of BDNF was decreasing in patients with depression and severe tinnitus. However, the relationship between the incidence of tinnitus and the role of BDNF still remain unclear in auditory cells. The aim of this study is to consider the molecular network of tinnitus focusing on BDNF signal and autophagy in auditory cells.

Methods: We used auditory cell line (HEI-OC1) in this study. Tunicamycin was used as Endoplasmic Reticulum (ER) stress. The viability of HEI-OC1 was determined by cell viability assays. Morphological observation was performed under Transmission electron microscope (TEM). Western blot analysis was performed.

Results: The cell viability after treatment of Tunicamycin in HEI-OC1 was decreased in dose- and time-dependent manner. Autophagosomes and autolysosomes were confirmed in the cytoplasm of HEI-OC1 under TEM. The expression of LC3-II was increased in HEI-OC1 after treatment of Tunicamycin. These results mean that autophagy was consistently induced in Tunicamycin-treated cells. The expression of CHOP was decreased after peaking at 12 hours after the treatment of Tunicamycin. These results lead to the suggestion that autophagy determined auditory cell death.

Discussion: The induction of CHOP in Tunicamycin-treated HEI-OC1 cells is attributed to the AKT/TSC/mTOR pathway. We now discuss molecular mechanisms for development of tinnitus from the standpoint of the autophagy-centered regulation of cell death in auditory cells under ER stress.

METHODS AND MATERIALS

Cell line: HEI-OC1 (House Ear Research Institute; now UCLA)
ER stress: Tunicamycin (5μg/ml, 75μg/ml, and 100μg/ml) (Sigma)
Cell viability assay: Cell viabilities were calculated with CountessTM. In Vitro, Life Technologies, USA after stain with trypan blue.
Western blot analysis: Antibody against LC3 was from MBL. Antibody against BDNF, TrkB, Bcl-2 and Beclin1 were from Santa Cruz BioTec. Antibody against CHOP and CAPS2 were from Cell Signaling. Antibody against Math1, Myosin 7a and Nestin were from Cell Signaling. Antibody against β-actin was purchased from Bio Legend.
Transmission Electron Microscope: Cells were fixed for 30 min with ice-cold 3% glutaraldehyde in 0.1M cacodylate buffer, embedded in Epon, and processed for transmission electron microscopy by standard procedure.

RESULTS

Fig.1. The cell viability after treatment of Tunicamycin in HEI-OC1 cells HEI-OC1 treated with different concentrations of Tunicamycin for 0-48h exhibited dose-and time-dependent cell death.

Fig.2. The expression of CHOP after treatment of Tunicamycin in HEI-OC1 cells The expression of CHOP was reduced after peaking at 12h in Tunicamycin treated cells.

Fig.3. The induction of LC3-II after treatment of Tunicamycin in HEI-OC1 cells Tunicamycin treatment result in time-dependent accumulation of LC3-II.

Fig.4. The morphological change after treatment of Tunicamycin under TEM A small number of autophagosome (blue arrow) were accumulated at 24 h in Tunicamycin-treated cells. At the point of 48 h, some autophagosomes eventually merged with lysosomes to become autolysosomes (black arrow).

Fig.5. The expression of Bcl-2 and Beclin1 after treatment of Tunicamycin in HEI-OC1 cells The expression of Bcl-2 which control apoptosis and Beclin1 that regulate autophagy were decreased in Tunicamycin treated HEI-OC1 cells.

Fig.6. The expression of BDNF and CAPS2 after treatment of Tunicamycin in HEI-OC1 cells The expression of BDNF which has the potential to become a biomarker of tinnitus and Calcium-dependent activator protein for secretion 2 (CAPS2) which promote BDNF section were decreased in Tunicamycin treated HEI-OC1 cells.

Fig.7. The expression of TrkB after treatment of Tunicamycin in HEI-OC1 cells The expression of TrkB was reduced after peaking at 12h in Tunicamycin treated cells.

Fig.8. The expression of inner ear marker in Tunicamycin-treated HEI-OC1 cells The expression of Math1, Myosin 7a and Nestin were reduced in time-dependent manner in Tunicamycin-treated HEI-OC1 cells.

CONCLUSIONS

Our results lead to the suggestion that the autophagy-mediated regulation of cell death and BDNF signaling pathway could play a major part of the incidence of tinnitus in cell level.

REFERENCES

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