Purpose Our aging society leads to an increasing incidence of neurodegenerative diseases. To date, the development of defined therapies has been hampered because the pathological mechanisms are poorly understood. Cell-based additive gene therapies to enhance the expression of protective factors are considered a promising modality for the treatment of neurodegenerative diseases, such as age-related macular degeneration (AMD). We have developed a method to stably overexpress the genes encoding pigment epithelium-derived factor (PEDF) and brain-derived neurotrophic factor (BDNF) into the genome of primary human retinal pigment epithelial (RPE) cells by electroporation using the Sleeping Beauty (SB) transposon system. BDNF is the most abundant neurotrophin in the central nervous system. PEDF is a multifunctional protein with anti-angiogenic and neurotrophic properties.

Methods Primary RPE cells were isolated from various human donor eyes and maintained individually in culture. After reaching confluence, RPE cells were trypsinized and co-transfected in suspension with two plasmids encoding SB100X transposase and the transposon carrying a PEDF and BDNF transcription cassette, respectively. The results of transfection were evaluated by different methods including microscopy, immunoblotting, ELISA, and quantitative PCR (qPCR).

Results Seeding of sufficient numbers of primary human RPE cells allows cultivation and growth into an integrated monolayer of pigmented, hexagonally shaped cells, independent of the donor age (65.3 ± 9.94 a, min: 49 a, max: 83 a, n = 12), post-mortem time of isolation (37.3 ± 17.0 h, min: 16 h, max: 68 h), and cultivation time (27.6 ± 14.1 d, min: 13 d, max: 61 d). Successful transfection was demonstrated in experiments performed independently. Applied electrical pulses had no negative effects on cell morphology. Gene expression of PEDF and BDNF was significantly increased compared with non-transfected control cells. Secretion of recombinant PEDF and BDNF proteins was also significantly elevated and remained stable over time.

Conclusion The studies using primary human RPE cells are an important step in the development of a cell-based PEDF or BDNF gene therapy that could be applied as an advanced therapy medicinal product to treat AMD or other degenerative retinal diseases.