MADGIC
Generation of Improved Cellular and Animal Models for Identification of Disease Phenotype and New Therapeutic Targets of Alzheimer's Disease

Area/ disease Entity: Advanced experimental models of neurodegenerative diseases/ Alzheimer's Disease
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Aims
Alzheimer's disease (AD) is a neurodegenerative disorder, which progressively and irreversibly affects different areas of the brain that are involved in learning and memory. While altered physiological processes of AD have been intensely investigated, efficient treatments halting or slowing the cognitive deficits are lacking. This is mainly due to lack of relevant research models that closely recapitulate brain environment and shows all aspects of AD pathology. We aimed at generating robust and advanced *human* cellular models that circumvent the limitations of current models of AD, helps to understand disease processes and identify new pertinent therapeutic targets. Exploiting genuine human based disease models are likely to help tackling the societal challenge of neurodegenerative diseases, including AD, by providing relevant research material for understanding the disease mechanisms and revealing potential druggable targets and biomarkers.

Means/methods
We generated patient-derived induced pluripotent stem cells (iPSCs) from individuals carrying mutations (PS1 and APP) leading to familial AD, and carriers of APOEε4/4 alleles. Healthy control lines and PS1 isogenic lines were generated as well. We established and optimized differentiation protocols for cortical and hippocampal neurons, astrocytes (iAstros), microglia (iMG), brain endothelial cells, cerebral organoids (CO) and hippocampal spheroids (HCS) containing hippocampal granule cells, and set up methods for engrafting iPSC-derived microglia into CO or immunodeficient mice, and for transplanting iMGs, iAstros or HCS into immunodeficient mice. We also set up 3D cell culture models and analysed cell-to-cell trafficking of extracellular vesicles involving inflammatory miRNAs.

Major findings
We found increased intracellular and extracelluar Aβ42/40 ratio in the mutated cortical and hippocampal neurons. This was also true for AD HCSs that additionally exhibited increased phosphorylated Tau (37 KDa), together with a decrease in pre-synaptic proteins, and altered neurite branching and neuronal excitability. Proteomic and metabolomics approaches demonstrated the presence of alterations in cellular network of AD HCSs. Moreover, a prion-like Aβ aggregation can be induced in AD neurons. iAstros showed robust phenotype, including increased release of Aβ42, altered cytokine release, dysregulated Ca^{2+} homeostasis and mitochondrial metabolism, increased oxidative stress and reduced lactate secretion, as well as compromised neuronal supportive function, as evidenced by impairing Ca^{2+} responses in healthy neurons, indicating that neuronal dysfunction in AD can be triggered by astrocytes. Additionally, astrocyte aberrancies were enhanced by immunostimulation. These pathologies were largely corrected by increased activation of Nrf2 transcription factor by gene therapy or small molecules. PS1 mutant astrocytes also had impaired fatty acid oxidation (FAO), which could be rescued by activation of the PPARβ/δ signalling in cultured astrocytes. Treatment with PPARβ/δ

signalling activators improved cognitive deficits observed in APP/PS1 model of the disease. Neonatal transplantation of AD iAstros also altered cognitive function of recipient mice.
We demonstrated that iMGs support neuronal maturation in COs. APOEε4/4 gene but not APP or PS1 mutation triggered severe alterations of iMGs critical functions (clearance, migration, release of inflammatory molecules). We also observed deregulated cell-to-cell trafficking mediated by extracellular vesicles involving inflammatory miRNAs (e.g. miR-124, miR-155, miR-21, miR-125b and miR-146a) from iPSC-derived neurons, iAstros, HCSs, and COs.
The blood-brain barrier model consisting of iPSC-derived brain endothelial cells (iBECs) showed that AD-iBECs exhibit altered tight and adherent junction protein expression, as well as efflux properties.

Impact and challenges: Our improved cellular and animal models reveal novel cell specific phenotypes, which depend on gene mutations and genetic risk factor contributing to AD. The models demonstrate disease mechanisms that can be therapeutically targeted by small molecules or gene therapy and serve as promising tools for early diagnosis, prevention and personalized medicine. As AD pathogenesis appears to largely depend on genetic background, a major challenge is to generate and validate iPSC-based models for AD cases that are affected by a wide variety of genetic and epigenetic factors.