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PERSPECTIVE

Synthetic cell pathobiology to study neurodegeneration: defining new therapeutic targets in astroglia

Synthetic biology is the design and construction of new biological parts, devices, and systems, and the re-design of existing, natural biological systems for useful purposes. This new interdisciplinary effort has gained interest from the industry sector, and synthetic biology has been used to produce fragrances, such as vanillin in yeast, in cells that do not normally exist in nature (Carlquist et al., 2015). Moreover, to simplify the process of developing new drugs, which requires many years and significant resources, testing in animal disease models could be replaced by engineered cells that represent and underlie the function of an organ. High content omic techniques in combination with stable human *in vitro* cell culture systems can be used to improve pre-clinical safety regimes by providing detailed mechanistic information on altered cellular processes. In cultured human renal epithelial cells, the mechanism of action of the nephrotoxin cyclosporine A was quantified by liquid chromatography-tandem mass spectrometry for kinetic modeling, and this approach appeared useful in determining the key biological properties of epithelial cells such as cell metabolism and the proteins required for the formation of proximal tubule epithelia (Aschauer et al., 2013; Wilmes et al., 2013).

Can synthetic biology be used to define molecular mechanisms and new potential therapeutic targets underlying neurodegeneration? The limited arsenal of cures for neurologic diseases reflects a fundamental problem of ill-defined cellular pathobiology of neurodegenerative diseases. Cell-based therapies using stem cells are developed on the widespread assumption that neurons are the sole elements in neurophysiology and neuropathophysiology, with synapses and neurotransmitter receptors as the chief regulatory elements in neuronal networks (Verkhratsky and Parpura, 2016). In contrast, neurodegenerative diseases may begin as a failure in neuroglia, which constitutes a diverse non-neuronal cell population and maintains multifaceted brain homeostasis, and can be envisioned as the pivotal element in neurologic or psychiatric diseases (Verkhratsky and Parpura, 2016).

To demonstrate that the origin and/or progression of neurodegenerative diseases is associated with functional changes in astrocytes, an abundant glial cell type, two major challenges have to be overcome; first, astroglial cells must be obtained in sufficient quantities (from diseased and/or aged-matched healthy individuals from families with disease history) by inflicting minimal damage and discomfort to donor persons; and second, a robust and reliable testing system is required allowing accurate measurement of mutated gene-encoded dysfunction affecting homeostatic performance of astroglia *in vivo*. The solution to the first problem was recently provided by Caiazzo et al. (2015), who used direct cell-reprogramming technology based on the dominant action of cell-lineage transcription factors and succeeded in identifying three transcription factors, nuclear factor I/A (NFIA), nuclear factor I/B (NFIB), and SOX9 (from 14 tested) that were sufficient to convert embryonic and postnatal mouse fibroblasts into induced astrocytes (iAstrocytes). By applying a relatively fast protocol (~2 weeks), iAstrocytes were generated by individually cloning coding regions of NFIA, NFIB, and SOX9 into doxycycline (dox)-inducible lentiviral vectors, and each lentivirus was used to infect mouse fibroblasts. iAstrocytes were comparable with native brain astrocytes as confirmed by gene-expression profiling and by functional tests. By analogy, a similar protocol could be applied to generate functional iAstrocytes from human fibroblasts in family members with documented medical history of neurodegenerative diseases, *i.e.* autosomal dominant Huntington's disease, familial amyotrophic lateral sclerosis and familial Alzheimer disease (AD), all progressive, irreversible, and ultimately fatal neurodegenerative diseases, in which a loss of astroglial homeostatic capabilities may play a key role in pathogenesis (Verkhratsky and Parpura, 2016). Cultured human iAstrocytes maintained in a dish devoid of a pathologic tissue environment may thus represent an alternative cell-based system enabling identification of astrocyte-specific processes associated with neurodegenerative disease. This approach is of value, because it provides the means to avoid misinterpretation of astroglial dysfunction as a secondary (adaptive or compensatory) response to putative primary neuron-derived insult.

To examine whether familial AD pathology is accompanied, from the very early disease stage, by a failure in astroglia function, we monitored vesicle traffic in cultured astrocytes from wild-type (wt) and a mouse model of AD (3xTg-AD) in the pre-symptomatic phase of the disease (Stenovec et al., 2016). The 3xTg-AD mouse model harbors mutant genes for amyloid precursor protein (APPSwe), presenilin 1 (PS1M146V), and microtubule-associated protein Tau (TauP301L), and mimics the spatiotemporal progression of amyloid and Tau protein pathology as in human AD (Stenovec et al., 2016). In this AD model, astrocytes express only a mutant presenilin-1 (PS1M146V), whereas neurons express in addition mutated amyloid precursor protein (APP) and Tau. Hybrids 129/C57BL6 from the same strain and genetic background as the presenilin 1 (PS1M146V) knock-in mouse, but with the endogenous wild-type mouse PS1 gene (wt), were used as controls. Astrocytes from both mice were maintained in culture to prevent exposure to the pathologic tissue environment associated with long-term accumulation of Aβ, neuroinflammatory response or a compromised blood-brain barrier.

In this animal model, astrocytic morphological atrophy was reported (Olabarria et al., 2010), which may reflect altered subcellular vesicle dynamics. Therefore, the dynamics of peptidergic secretory vesicles and acidic organelles (endolysosomes) were studied in living astrocytes by labeling vesicles with a plasmid encoding atrial natriuretic peptide tagged with mutant green fluorescent protein (ANP.emd) and by LysoTracker dye (LyTR), respectively, and using quantitative fluorescence imaging (**Figure 1A**). The results revealed that the tracks of peptidergic vesicles were more elongated in wt than in 3xTg-AD astrocytes, indicating reduced directional vesicle mobility in 3xTg-AD astrocytes (**Figure 1B, C**). Moreover, vesicle speed was reduced by ~24% in 3xTg-AD versus wt cells (**Figure 1D**, left). A similar result, although much smaller in amplitude, was obtained when studying acidic organelles (**Figure 1D**, right), which also contain gliosignaling molecules, such as ATP, and also receptors, channels, and transporters (Vardjan et al., 2015). Overall, in comparison with wt astrocytes, the spontaneous mobility of peptidergic and acidic secretory vesicles was substantially diminished in 3xTg-AD astrocytes, which may contribute to the morphological alterations seen in histopathologic examinations of neurodegeneration (Olabarria et al., 2010). Importantly, disproportionally affected vesicle trafficking in peptidergic and acidic vesicles indicates a gross imbalance in vesicle dynamics. This may alter the cell surface signaling landscape, because secretion of peptides was reduced in 3xTg-AD astrocytes versus wt controls (Stenovec et al., 2016), and cell shape dynamics on a longer timescale, and the proper distribution of proteins, including APP and Aβ during neurodegeneration. The clearance of Aβ is facilitated by extracellular proteolysis, export across the blood–brain barrier, and cellular uptake. Astrocytes take up and degrade Aβ, but diminished mobility of endolysosomes (Stenovec et al., 2016), the major degrading organelles, wherein Aβ localizes after uptake, may insufficiently support effective clearance of waste in the glymphatic system (Thrane et al., 2014) and thus contribute to the development of amyloid plaque pathogenesis over a longer time.

The decreased instantaneous speed in relatively fast-moving peptidergic vesicles may indicate that vesicles were arrested more frequently along the cytoskeleton or were less effectively dragged by the motor proteins during processive motor "walking" along the microtubules. In accordance with the abovementioned possibility,

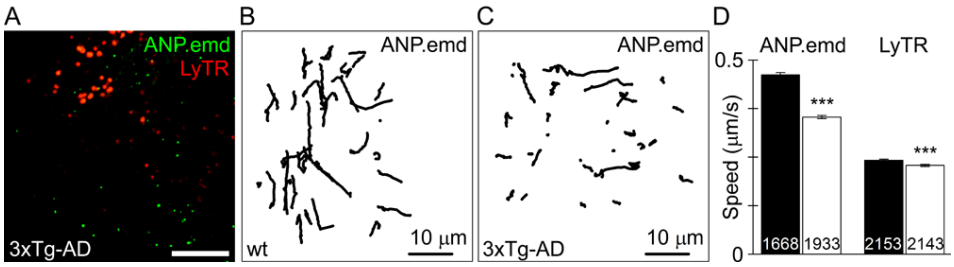

Figure 1 Attenuated mobility of peptidergic (ANP.emd) and acidic vesicles in 3xTg-AD astrocytes.
(A) A double fluorescent confocal image of the 3xTg-AD astrocyte expressing ANP.emd stored in individual vesicles observed as bright green fluorescent puncta and LysoTracker-labeled (LyTR) vesicles observed as red fluorescent puncta; scale bar: 10 μm. (B) Vesicle tracks ($n = 45$) obtained in a 15-second epoch of imaging representative control (wt) and (C) 3xTg-AD astrocytes expressing ANP.emd. Note less elongated vesicle tracks in the 3xTg-AD astrocyte. (D) Speed of ANP-loaded vesicles and LyTR-labelled vesicles in wt (black bars; mean ± SEM) and 3xTg-AD astrocytes (white bars). Note substantially diminished speed of peptidergic vesicles and modestly diminished spee of LyTR-labeled vesicles in 3xTg-AD astrocytes. The numbers at the bottom of the bars indicate the number of vesicles analyzed. ***$P < 0.001$, *vs.* wt (Mann-Whitney *U* test).

four times more pauses were observed in 3xTg-AD astrocytes than in wt astrocytes (Stenovec et al., 2016). Altered vesicle trafficking in PS1M146V–expressing astrocytes originates from mutant PS1 (characteristic for the early-onset familial AD), which may alter microtubules associated motor protein activity by their phosphorylation *via* GSK3β whose activity is increased in the presence of PS1M146V. Concomitant with an increased GSK3β activity, increased relative levels of kinesin light chains phosphorylation and the reduced amount of kinesin-1 bound to membranous organelles were observed in cultured cells expressing PS1M146V (Pigino et al., 2003). Changes in vesicle dynamics in 3xTg-AD mouse astrocytes indicate that this cellular process may also represent the therapeutic target in some neurologic conditions. Indeed, vesicle mobility was decreased (Vardjan et al., 2015) by fingolimod (FTY720), a drug that has been recently introduced for the treatment of multiple sclerosis (Trkov et al., 2012). It was shown that FTY720 accumulates in the white matter in the central nervous system, where it can reach concentrations that affect astrocytic vesicle mobility and consequently their ability to participate in regulated exocytosis. This action may be part of its therapeutic efficacy in patients with multiple sclerosis, a condition where neuroinflammation involves endolysosomal vesicle traffic and antigen presentation (Vardjan et al., 2015). The mechanism of reduction of vesicle mobility by fingolimod likely involves fingolimod-induced changes in $[Ca^{2+}]_i$ homeostasis, which impair all types of vesicles tested. Thus, new therapeutics, such as FTY720, that affects vesicle mobility represent a novel possibility for the treatment of neurologic diseases, including neurodegeneration, where a disproportionate mobility attenuation of distinct vesicle types was observed (Stenovec et al., 2016).

In summary, experiments on 3xTg-AD mouse astrocytes, devoid of their pathologic environment, revealed, for the first time, that the expression of mutated presenilin 1 (PS1M146V) differentially alters the dynamics of different vesicle types, which may contribute to the development of AD (Stenovec et al., 2016). The same experimental approach, however, is not possible in humans. Here, the use of iAstrocytes represents the major technological advancement and the only acceptable alternative to experimentally address the early dysfunction in cultured astroglial cells converted from fibroblast of diseased (and healthy) members of families with medical history of neurodegenerative diseases. Human iAstrocytes can be further used to develop a new diagnostic test based on analysis of vesicle mobility, which may aid predict the clinical manifestation of the disease already in the early, pre-symptomatic phase of disease. Thus, the synthetic pathobiology approach, where cell-reprogramming technology is used to convert embryonic, postnatal or adult fibroblasts, isolated from a patient, into induced astrocytes (iAstrocytes), appears to be a promising strategy to identify new mechanisms and targets in astroglia associated with neurodegeneration, such as AD.

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References

Aschauer L, Gruber LN, Pfaller W, Limonciel A, Athersuch TJ, Cavill R, Khan A, Gstraunthaler G, Grillari J, Grillari R, Hewitt P, Leonard MO, Wilmes A, Jennings P (2013) Delineation of the key aspects in the regulation of epithelial monolayer formation. Mol Cell Biol 33:2535-2550.

Caiazzo M, Giannelli S, Valente P, Lignani G, Carissimo A, Sessa A, Colasante G, Bartolomeo R, Massimino L, Ferroni S, Settembre C, Benfenati F, Broccoli V (2015) Direct conversion of fibroblasts into functional astrocytes by defined transcription factors. Stem Cell Reports 4:25-36.

Carlquist M, Gibson B, Karagul Yuceer Y, Paraskevopoulou A, Sandell M, Angelov AI, Gotcheva V, Angelov AD, Etschmann M, de Billerbeck GM, Liden G (2015) Process engineering for bioflavour production with metabolically active yeasts - a mini-review. Yeast 32:123-143.

Olabarria M, Noristani HN, Verkhratsky A, Rodríguez JJ (2010) Concomitant astroglial atrophy and astrogliosis in a triple transgenic animal model of Alzheimer's disease. Glia 58:831-838.

Pigino G, Morfini G, Pelsman A, Mattson MP, Brady ST, Busciglio J (2003) Alzheimer's presenilin 1 mutations impair kinesin-based axonal transport. J Neurosci 23:4499-4508.

Stenovec M, Trkov S, Lasic E, Terzieva S, Kreft M, Rodriguez Arellano JJ, Parpura V, Verkhratsky A, Zorec R (2016) Expression of familial Alzheimer disease presenilin 1 gene attenuates vesicle traffic and reduces peptide secretion in cultured astrocytes devoid of pathologic tissue environment. Glia 64:317-329.

Thrane AS, Rangroo Thrane V, Nedergaard M (2014) Drowning stars: reassessing the role of astrocytes in brain edema. Trends Neurosci 37:620-628.

Trkov S, Stenovec M, Kreft M, Potokar M, Parpura V, Davletov B, Zorec R (2012) Fingolimod--a sphingosine-like molecule inhibits vesicle mobility and secretion in astrocytes. Glia 60:1406-1416.

Vardjan N, Verkhratsky A, Zorec R (2015) Pathologic potential of astrocytic vesicle traffic: new targets to treat neurologic diseases? Cell Transplant 24:599-612.

Verkhratsky A, Parpura V (2016) Astrogliopathology in neurological, neurodevelopmental and psychiatric disorders. Neurobiol Dis 85:254-261

Wilmes A, Limonciel A, Aschauer L, Moenks K, Bielow C, Leonard MO, Hamon J, Carpi D, Ruzek S, Handler A, Schmal O, Herrgen K, Bellwon P, Burek C, Truisi GL, Hewitt P, Di Consiglio E, Testai E, Blaauboer BJ, Guillou C, et al. (2013) Application of integrated transcriptomic, proteomic and metabolomic profiling for the delineation of mechanisms of drug induced cell stress. J Proteomics 79:180-194.