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, and cell neuropheromone receptor as in the Caenorhabditis (Carlsberg 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 conflicting 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 cell-reprogramming technology based on the dominant 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).

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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 $[Ca^{2+}]$, homeostasis, which impair all types of vesicles tested. Thus, new therapeutic strategies, 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 fibroblasts 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 astrogliosis associated with neurodegeneration, such as AD.

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Matija Stenovec, Robert Zorec* Celica Biomedical, Ljubljana, Slovenia Laboratory of Neuroendocrinology-Molecular Cell Physiology, Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

*Correspondence to: Robert Zorec, Ph.D., robert.zorec@mf.uni-lj.si.
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