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COMMENT

Mapping the hotspots for DNA repair synthesis in human brain organoids

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Even stored carefully and properly inside the cell nuclei, DNA is constantly attacked by endogenous and exogenous agents, causing surprisingly extensive DNA damage. The organisms have evolved DNA repair systems to cope with those potentially deleterious lesions [1]. The human nervous system is formed through the extensive proliferation of the neural stem cells that gradually exit from the cell cycle, migrate, and finally become longest-living "postmitotic" neurons at their destinations [2]. The DNA repair mechanism relying on homologous genetic recombination, which is operated during the S/G2 phase of the cell cycle, might be absent in the postmitotic cells, such as neurons [3]. At the same time, taking into consideration that the brain is thought to metabolize as much as a fifth of consumed oxygen, base lesions in the DNA generated by the attack from reactive oxygen species (ROS) over time are particularly serious for long-living neurons. Those are generally repaired by the base excision repair (BER) pathway [3]. ROS may also lead to form DNA single-strand breaks (SSBs) and double-strand breaks (DSBs), but SSBs might be crucial for postmitotic neurons [4]. These repair types of machinery are essential in the nervous system as the defect in the DNA repair system results in neurodegenerative disease or neurodevelopmental abnormality [3, 5]. However, the identity of DNA damages accrue and their distribution throughout the genome of postmitotic neurons remained elusive. Two studies independently appeared in the recent issues of *Science* [6], and *Nature* [7] now delineate the identity of the lesions and map them in the genome of postmitotic neurons.

The core of works reported by Reid et al. [6] and Wu et al. [7] is a sequencing technique to map the sites of DNA repair synthesis in the genome by capturing the non-replicative incorporation of nucleoside analog 5-ethynyl-2′-deoxyuridine (EdU). The methods are designated as Repair-seq and synthesis associated with repair sequencing (SAR-seq) by Reid et al. and Wu et al., respectively. They applied their new method to human embryonic stem cell-induced neurons (ESC-iNs) and postmitotic glutamatergic neurons derived from induced pluripotent stem cells (iPSCs) (i^{3}Neurons). Wu et al. also observed SAR-seq peaks in primary rat neurons ruling out the possibility of an artifact of iPSC differentiation. On the other hand, Wu et al. did not detect incorporation of EdU in skeletal muscle cells differentiated from iPSCs (iMuscle) or G0-arrested pre-B cells. Both studies reached the same conclusion that postmitotic neurons accumulate an unexpectedly high level of recurrent repair synthesis at neuronal enhancers of genes required for identity and functionality. Wu et al. further uncovered the SAR-seq peaks coincide with the reads of ChIP-seq for ADP-ribose and XRCC1, indicating that SSBs are the causative lesions of recurrent repair synthesis. Wu et al. also devised a technique designated as S1 END-seq by modifying previously reported END-seq, which enabled locating endogenous SSBs with much higher resolution (at single-nucleotide resolution) than did SAR-seq and demonstrated that CpG dinucleotides were highly enriched at SAR sites.

While featuring the scientific novelty achieved by Reid et al. and Wu et al., we would like to provide perspectives, mainly focusing on the "human brain organoids" (one can find excellent reviews regarding the cutting-edge technology in the ***Special Issue*** of this journal; https://www.nature.com/collections/ihjdghgcfb).

DIVERSE NEURONAL SUBTYPES DIRECTED BY TRANSCRIPTION FACTORS (TFS) AND THE EPIGENETIC LANDSCAPE

Both Reid et al. and Wu et al. induced postmitotic neurons by forced expression of a single gene, *NEUROD1*. However, as the transcriptomic analysis of neurons in the human brain at a single cell (nucleus) level, i.e., single-cell RNA-seq (scRNA-seq), revealed unexpected heterogeneity and diversity of neuronal subtypes [8], diverse sets of TFs that drive neuronal cell fate should exist. Indeed, in mice, it has been shown that certain groups of TFs can induce fibroblasts (MEFs) to gain a neuron-like identity in vitro, and those cells generated by TF-directed differentiation share a "core" cell-autonomous neuronal signature with endogenous neurons in vivo [9]. In humans, pooled CRISPR activation screens identified factors that regulate neuronal fate specification of human pluripotent stem cells (PSCs). Thus, it would be intriguing to explore whether the hotspots of repair synthesis in different types of human postmitotic neurons induced by different sets of TFs have an identical pattern or otherwise there are neuron-type specificities. As illustrated along with the flow to the right side in Fig. 1, each type of neuron is formed through different paths of epigenetic landscape and, consequently, has a different epigenetic signature. Therefore, different types of neurons might have different cycles of cytosine methylation and demethylation [10]. Aligning the profiles of repair sites for different kinds of neurons would give more fundamental insights regarding the role of SSBs

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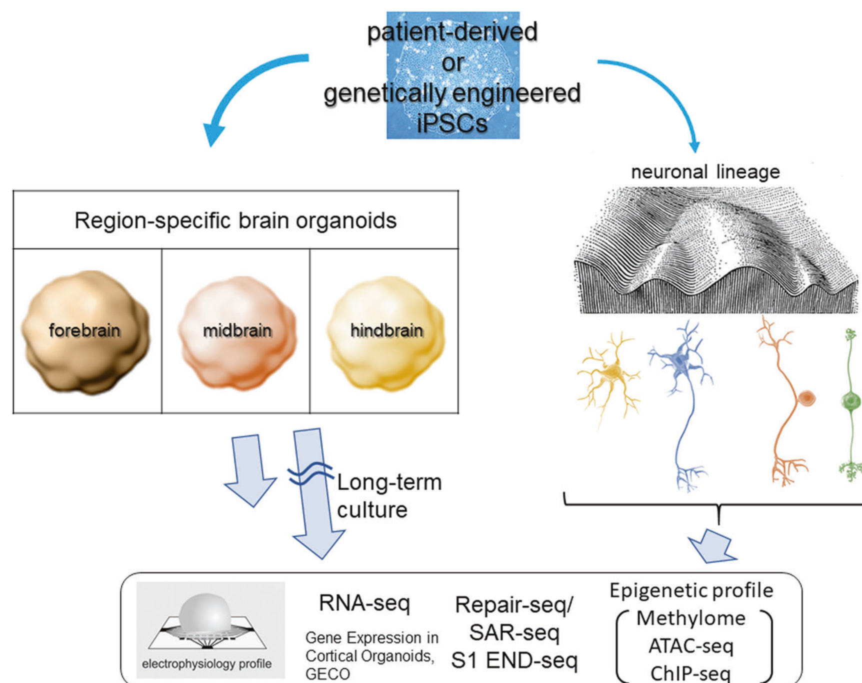

Fig. 1 Future directions of research following the reports of Reid et al. and Wu et al. (*Flow to the right side*) In addition to *NEUROD1*-induced neurons, other types of neurons might be analyzed. (*Flow to the left side*) Region-specific brain organoids are generated and analyzed. By modulating the length of the culture period, brain organoids have been shown to reach postnatal stages. With genetic engineering technology, brain organoids may mimic later stages of development. Both sides of studies may use iPSCs of healthy individuals and those of patients with DNA Repair deficiencies or isogenic lines mimicking the mutation of patients generated by gene-editing technology.

related to the cycle of cytosine methylation/demethylation during neuronal lineage specification.

FROM TRADITIONAL 2D TO 3D CULTURES

Directing iPSCs or ESCs to differentiate towards the neuronal lineages or directly transdifferentiating somatic cells into the neuronal cells with TFs has brought novel insights on neurogenesis and neurodegeneration. Nevertheless, the cells cultured in two-dimensional (2D) conditions are grown on an artificial dish and lack the brain's complex structural arrangement. Furthermore, 2D culture cannot capture in vivo human brain development, which is an extraordinarily complex process and relies on elaborate spatio-temporal regulation of signaling and gene expression. Over the past years, conceptual and technical advances to complement the mentioned limitations of 2D culture have been achieved by three-dimensional (3D) culture of stem cells to generate brain organoids [11]. Taking advantage of this sophisticated technology, it is now possible to model human neurodevelopmental and neurodegenerative diseases in vitro [12, 13]. In addition, brain organoid technology can be applied to understand the identity, distribution of DNA damage, and its relation to neurological diseases in the human brain.

CELL TYPE SPECIFICITY VS. BRAIN REGION SPECIFICITY

As highly parallel single-cell transcriptomics has underpinned the molecular basis of diversity of cell types, cell states, lineage progression, and network dynamics of brain organoids [11, 13], examining the Repair-seq/SAR-seq and S1END-seq in the brain organoids would clarify not only the cell type but the brain region specificity of DNA damage accrual.

REPAIR SYNTHESIS IN THE CONTEXT OF NEURONAL NETWORK DEVELOPMENT

From the viewpoint of functionality, brain organoids have shown to exhibit periodic and highly regular nested oscillatory network events that transitioned to more spatiotemporally irregular patterns, and the synchronous network events resembled features similar to those observed in preterm human electroencephalography [14]. Thus, integrating this approach with repair kinetics would shed light on the relationship between DNA repair and electrophysiological activity in the context of human neuronal network development.

MODELING WITH HUMAN CELLS

Defects in DNA repair mechanisms result in neurodevelopmental disorder or neurodegeneration [3, 5]. To explore the molecular and cellular basis underlying the neurological abnormalities exhibited by the patients, genetically engineered mouse has been used for disease modeling. However, mouse models do not fully capture the patient's neurological symptoms [3], which is natural considering the striking differences between humans and mice in brain development [13]. Brain organoids prepared with patient-derived iPSCs or iPSCs genetically engineered with gene-editing technology to harbor the same mutation as the patient are potentially helpful and would lead to more accurate modeling. In this regard, it would be essential to examine the cerebellar organoids generated from iPSCs capturing *XRCC1*-deficient patients. It has been reported that the mutant mouse, $Xrcc1^{Nes\text{-}Cre}$, mimicked the patient's symptoms, i.e., loss of cerebellar neurons and ataxia [15], despite the difference in neural progenitor populations of the cerebellum between human and mouse [16].

DNA REPAIR IN NEURODEGENERATIVE AND AGING DISEASE

DNA damage has been well acknowledged as central to aging, and genetic defect in DNA repair mechanism is associated with premature aging [17]. As neurodegeneration is the typical pathology of aging, and, indeed, some of the diseases deficient in DNA repair do exhibit age-associated neurodegenerative symptoms such as ataxia with oculomotor apraxia 1 (AOA1) and spinocerebellar ataxia with axonal neuropathy 1 (SCAN1). So, it is also possible to study the molecular and cellular mechanisms underlying those neurodegenerative diseases by using brain organoids generated from patient-derived or genetically engineered iPSCs. Although this reductionist model has several intrinsic limitations, human brain organoids are the only way to experimentally assess the neuronal diversity generated during neurodevelopment [18].

In summary, the research direction with brain organoids mentioned in this manuscript is illustrated along with the flow to the left side in Fig. 1. It is important to consider both results obtained with individual types of neurons (*right*) and brain organoids (*left*) in a well-balanced manner.

DATA AVAILABILITY

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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AUTHOR CONTRIBUTIONS

TK Jr. and ARM conceived of and wrote this paper.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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