Tongfei Liu

**Human stem cell-derived microglia will be an indispensable toolbox for Alzheimer’s disease research**

Alzheimer’s disease (AD) is the most common age-related dementia without cures. Between 2000 and 2017, deaths resulting from AD increased 145%. Based on the latest statistical data, more than 50 million people in the world suffered this devastating disease, which is one of major burdens in modern public health system (Alzheimer’s Association, 2020). Genetic mappings have uncovered numerous susceptibility genetic variants, the vast majority of which are located in noncoding regions of the genome.

Microglia, a type of glial cells, are resident macrophages located throughout the central nervous system. Vital functions of microglia within the brain are mainly related to innate immunity and maintaining homeostasis such as neuroinflammation, phagocytosis, brain environment surveillance, and synaptic pruning. Most of AD-risk variants are active in microglia (Nott et al., 2019). The enthusiasm for microglia was further fueled by the discovery that many of the microglia-enriched genes are identified as risk factors for AD, frontotemporal dementia and other neurodegenerative diseases.

However, current strategies and methodologies for studying its role in AD etiology and progression are limited. Firstly, prior knowledge about microglia mostly came from rodent models. Sixty-five million years of evolutionary divergence produce significant differences between human and rodents, during which their immune systems faced distinct pathogens. For example, TREM2 (Triggering Receptor Expressed on Myeloid Cells 2) and CD33 (Cluster of Differentiation 33), the focus of AD fields, also feature significant variability within their protein coding sequences, which may result in diverging biological differences between two species. The membrane-proximal immunoreceptor tyrosine-based inhibition motif (ITIM) found in human CD33 is absent in murine CD33. Moreover, a recent study showed that the TREM2 R47H mutation was observed to downregulate TREM2 mRNA levels in mouse microglia, but not in human. Thus, induced pluripotent stem cells (iPSCs) derived microglia-like model is one of the best choices currently. However, iPSC derived cell studies may be hindered by the lack of standardized reprogramming protocols and variables derived from genetic variation and epigenetic memory which affected results reproduction, data interpretation and integration (Kim et al., 2010). Therefore, it necessitates utilization of conventional human H9 embryonic stem (ES) cell line harboring AD-associated variants in AD research, which will be easier for following data validation and comparison between different laboratories.

Many labs have focused on singular profiles at transcriptomic or epigenetic levels. However, a big picture generated from epigenetic, transcriptomic, proteomic and functional profiles may be required to fully understand how AD-associated gene variants can affect microglia behavior.

In our recent published work, by CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)-Cas9 (CRISPR Associated Protein 9), we have integrated a subset of AD-associated genetic risk variants including TREM2 KO, TREM2 R47H, SORL1 (Sortilin Related Receptor 1) R744X and SORL1 A528T into an isogenic human H9 ES cell model, and differentiate H9 cells bearing those AD variants into mature human stem cell derived microglia-like cells (hMGLs) (Liu et al., 2020). Integrative multiomics will be generated and used to study and explore microglial behavior, namely TREM2 R47H, SORL1 KO variants can recapitulate microglial signatures observed in AD, upregulation of pathogenic components such as APOE found in these signatures may ultimately impair microglial function (Liu et al., 2020). But what will happen in long-term transplantation? Besides, data interpretation in current xenotransplantation paradigm will be confounded by endogenous mouse microglia (Hasselmann et al., 2019). A next-generation model will only contain the human microglia. Thus, it will be of interest to further study how Aβ cleared in AD patients, it is more suitable to use immunocompetent AD mouse lines for related studies. To facilitate hMGL xenotransplantation in immunocompetent AD mice model, based on recent work of Deuse et al. (2019) in Nature Biotechnology, genetic ablation of the major histocompatibility complex and overexpression of the transmembrane protein CD47 (Cluster of Differentiation 47) could make hMGL invisible to the immune system of the host.

The xenotransplantation experiments need different phenotypes of hMGLs, which is very important to new learner.

3) Both hypothesis-driven and data-driven ways are needed for AD research. Traditionally, most scientific research is conducted in a hypothesis-driven way, in which researchers submitted a specific, measurable, and testable question at the beginning, then tested with an experiment, analyzed the results and modified the hypothesis. Such a cycling proceeding in past centuries is the most reliable approach to produce robust knowledge. In contrary, for data-driven, there are no more hypotheses, no more discussions whether the experiments refute or support the original hypotheses. In this new era with big data technologies booming, we need to add the data-driven way into our research strategies, which utilized complex algorithms and statistical tools to go through a massive amount of AD clinical data to find useful information that could be turned into knowledge. For example, spatial transcriptomics (Visium-10xgenomics; STARmap) could collect very important spatial information in AD brain tissue samples, including the relationship of microglial cells to amyloid plaques/Tau tangles, in order to discover the potential changes in cell composition and gene expression distribution during neurodegeneration. Furthermore, it can also
4) Inducing aging effects in hMGLs model. Our hMGLs show many similar features to human microglia such as global expression profiles, and functional responses including Aβ phagocytosis, microglial depolarization kinetics, Ca²⁺ homeostasis, and cytokine release.

However, the hMGL model is still in its infancy stage and yet requires further development. Aging is the greatest known risk factor for AD that cannot be explained by amyloid hypothesis. It is challenging to recapitulate the effect of aging in cell culture system. Mutant Lamtin A, also called progerin, can cause premature aging syndrome Hutchinson-Gilford progeria (HGPS). Exogenous expression of progerin has been proven promising as one method to age neurons (Miller et al., 2013). Interestingly, the expression of progerin has also been observed in fibroblasts from normally aged donors. So, we can integrate tet-on inducible progerin into the genome of hMGL to study the aging impact on microglial functions in a regulatory manner. Besides, the deficiency of XPF (Xeroderma pigmentosum complementation group F or ERCC4) or ERCC1 (Excision Repair Cross Complementation Group 1), both DNA excision repair proteins, can also lead to the HGPS disease, which will worsen the DNA damages over time. So, by CRISPR-Cas9, we can make XPF-/- or ERCC1-/- hMGL to study the aging effects. An interesting speculation is that some of late-onset AD risk variants (especially, non-coding regions) may produce only observable effects in aging background. Using the progerin or XPF-/- or ERCC1-/- hMGL as standard, we can introduce other AD risk variants identified from late-onset AD patients into genome of these hMGL and systematically determine the functional significance of these genetic variants, which could elucidate the causal relationship between genetic and AD phenotypes.

Technically, it is worthwhile to manipulate the epigenome status to induce aging in the hMGL cell (my unpublished data). DNA methylation is a type of epigenetic modification that refers to the addition of methyl groups onto DNA at specific sites, which determine whether certain genes are switched on or off. The Horvath clock is a most widely-used and accurate algorithm and uses DNA methylation data to calculate age surprisingly well, generally calculating age to within 3 years of a person’s real age and also works for non-dividing cells like neurons (Horvath, 2013). Those discoveries inspired us that aging-like outcomes may be stimulated through manipulating epigenetic modification in the genome of cells. Since epigenetic changes are reversible, it is possible to target their origin to reverse them. Recently, emerging epigenome editing technology has been developed to modify epigenetic marks. There are chimeric proteins, composed of two parts: a DNA binding domain, allowing proteins to precisely target on specific genomic loci, and a catalytically inactive Cas9 (dCas9), to a domain comprising an epigenetic factor (TE1 or DNMT3a) that will modify DNA. Using these chimeric proteins, we can change the DNA-methylation status of Horvath clock’s loci in the genome of hMGLs into what old age looks like.

In conclusion, hMGLs are a scalable discovery platform for AD and other neurological disorders including Parkinson’s disease, amyotrophic lateral sclerosis, and Huntington’s disease. To provide a profound understanding of microglia’s role in AD etiology and progression, it will require interrogation using a variety of models and approaches, bridging scales from molecules to system, and investigating mechanisms underlying the entire life course (Figure 1). Thus, we should utilize emerging stem cell tools, multiple omics platforms, and novel animal models to identify causal genetic elements and unexpected regulatory pathways contributing to AD pathogenesis and progression that will finally lead to curative treatments. Moreover, genome-engineered microglia, like chimeric antigen receptor T cells (CART), can also be an efficient vehicle for delivering neuroprotective or regenerative molecules, which will be new directions for treating a variety of neurological diseases in the future.

References
Alzheimer’s Association (2020) 2020 Alzheimer’s disease facts and figures. Alzheimers Dement doi: 10.1002/alz.12068.
Deuse T, Hu X, Gravina A, Wang D, Tedashigli G, De C, Thayer WO, Wahl A, Olafsson J, Reichenberg A, RibMaker 2020. Laerier LL, Schreper S (2019) Hypomimimic genes of induced pluripotent stem cells evade immune rejection in fully immunocompetent allogeneic recipients. Nat Biotechnol 37:252-258.
Hasselmann J, Coburn MA, England W, Figueroa Velez DX, Kiani Shabestari S, Tu CH, McQuade A, Kolandhasothan M, Echeverria K, Claes C, Nakayama T, Azevedo R, Coufal NG, Han CZ, Cummings BJ, Davyhan H, Glass CK, Healy LM, Gandhi SP, Spittle RC, et al (2019) Development of a chimeric model to study and manipulate human microglia in vivo. Neuro 103:1016-1033.
Horvath S (2013) DNA methylation age of human tissues and cell types. Genome Biol 14:R315.
Kim K, DOI A, Wen B, Ng K, Zhao R, Cahan P, Kim J, Arvey MJ, Ji H, Ehrlich L, Yabuchi A, Takeuchi A, Cunff KC, Hongxiang H, McKinnon-Freeman S, Navieros G, Yoon TJ, Izarray RA, Jung N, Seita J, et al (2010) Epigenetic memory in induced pluripotent stem cells. Nature 467:285-290.
Liu T, Zhu B, Liu X, Zhang X, Yin J, Li X, Jiang L, Hodges AP, Rosenthal SB, Zhu L, Yancy J, McQuade A, Blurtorn-Jones M, Tanz RE, Huang TY, Xu H (2020) Multi-omic comparison of Alzheimer’s variants in human ESC-derived microglia reveals convergence at AP0E. J Exp Med 217:e20200474.
Miller JD, Ganat YM, Kishinevsky S, Bowman RL, Liu B, Ey M, Mandak PK, Vera E, Shin JM, Khra S, Taldone T, Fusaili N, Tomishima M, Kranik D, Milner TA, Ross DJ, Studer L (2013) Human iPSC-based modeling of late-onset disease via progerin-induced aging. Cell Stem Cell 13:691-705.
Murray CE, King A, Troakes C, Hodges A, Lashey T (2019) AP0E epsilon4 is also required in TREM2 R47H variant carriers for Alzheimer’s disease to develop. Neuropathol Appl Neurobiol 45:183-186.
Nott A, Holtman IR, Coufal NG, Schlachtetski JCM, Yu M, Hu R, Han CZ, Pena M, Xiao J, Wu Y, Keulen Z, Pasillas MP, O’Connor C, Nickl CK, Schafner ST, Shen Z, Risonan RA, Brewer JB, Gosselin D, Gonda DD, et al (2019) Brain cell type-specific enhancer-promoter interactome maps and disease-risk association. Science 366:1134-1139.
Speicher AM, Wiendl H, Meuth SG, Speicher AM, Wiendl H, Meuth SG, Pawlowski M (2019) Human iPSC-based modeling of late-onset disease via progerin-induced aging. Cell Stem Cell 13:691-705.
Alzheimer’s Disease Facts and Figures. Alzheimers Dement 26:1-142.

Human stem cell-derived microglia will be an indispensable toolbox for Alzheimer’s disease research. Neuro Regen Res 16(9):1770-1771.

Copyright license agreement: The Copyright License Agreement has been signed by the author before publication.

Plagiarism check: Checked twice by iThenticate.