Rapid Generation of O4⁺ Oligodendrocyte Progenitor Cells from Human Induced Pluripotent Stem Cells for Myelination Studies

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Abstract

Myelin production by oligodendrocytes is critical for efficient neuronal communication, and disruptions in myelination are fundamental to numerous central nervous system disorders. Oligodendrocyte progenitor cells (OPCs) derived from human induced pluripotent stem cells (hiPSCs) offer a powerful platform for high-throughput drug screening aimed at myelin repair and disease therapies. These stem cell-derived models not only overcome the limitations of using primary human oligodendrocytes but also enable researchers to study oligodendrocyte development and function in a controlled environment. To accelerate the identification of therapeutic compounds for demyelinating diseases, BrainXell developed an optimized protocol that reduces OPC differentiation time from three months to less than one month, yielding highly pure populations of OPCs. Quantitative analysis revealed a significant increase in MBP expression, with 60-80% of cells positive for MBP under optimized conditions. Flow cytometry demonstrated ~80% O4+ cells in the best differentiation condition, and qPCR showed upregulation of oligodendrocyte-related genes (CNP, OLIG2, CSPG4) as well as myelin associated markers (PLP1 and MBP). When co-cultured with cortical glutamatergic neurons for 14 days, MBP+ oligodendrocyte projections wrapped around MAP2+ neurites, indicating functional interactions between oligodendrocytes and neurons. This demonstrates the maturation of OPCs and their ability to myelinate in a neuronal environment. Both co-cultures and monocultures exhibited complex, arborized morphology, confirming OPC maturation and structural development under optimized conditions. The ability to rapidly and efficiently differentiate large quantities of hiPSC-derived oligodendrocytes, especially using optimized protocols, accelerates the discovery of drug candidates for myelin repair and regeneration. This system enables functional interactions between OPCs and neurons, making it an ideal platform for drug screening and myelin repair research. It provides a consistent and reproducible model for studying oligodendrocyte development and offers new opportunities for therapeutic screening that would be challenging with primary human oligodendrocytes, ultimately accelerating the development of treatments for disorders associated with myelin loss.

Oligodendrocyte Differentiation and Development

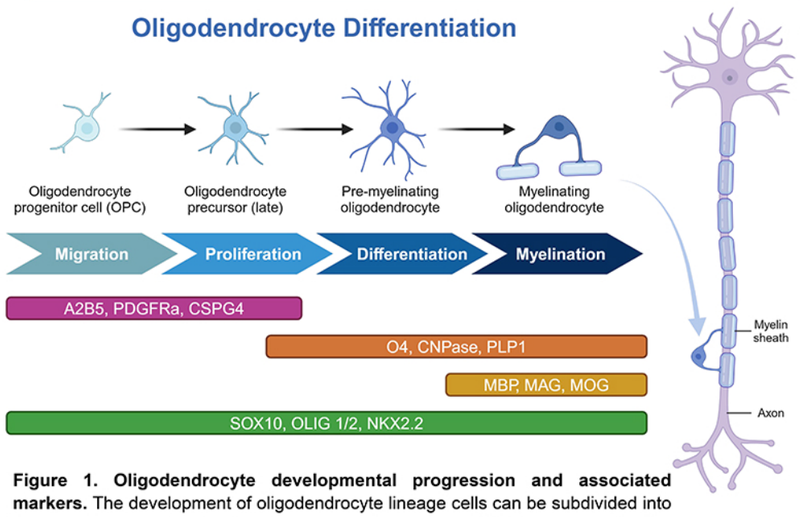


Figure 1. Oligodendrocyte developmental progression and associated markers. The development of oligodendrocyte lineage cells can be subdivided into three stages: oligodendrocyte progenitor cells (OPC), pre-myelinating oligodendrocytes and mature oligodendrocytes (OLs). Stages are identified by their increasingly complex morphology and expression pattern of well-defined markers.

Optimizing Oligodendrocyte Differentiation Conditions

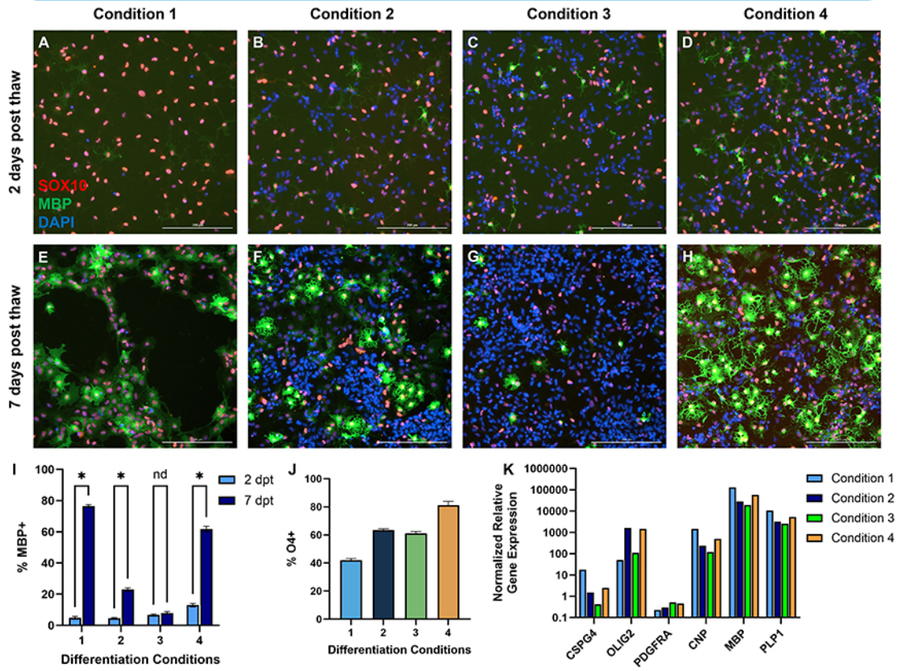


Figure 2. Optimizing Culture Conditions of Human Pluripotent Stem Cell Derived Oligodendrocyte Progenitor Cells. (A-H) OPCs were thawed and cultured for 2 or 7 days under four experimental conditions, followed by immunostaining for SOX10 (red) and MBP (green). (I) Quantitative analysis revealed a significant increase in MBP expression, with optimized conditions yielding MBP positivity in 60-80% of cells. (J) Flow cytometry analysis post-thaw demonstrated robust expression of the oligodendrocyte surface marker O4, with approximately 80% of cells positive for O4 in differentiation condition 4. (K) qPCR analysis revealed an upregulation of oligodendrocyte-related genes in all experimental conditions. Scale bars = 200µm (A-H).

Molecular Characterization of Human Glial Cells

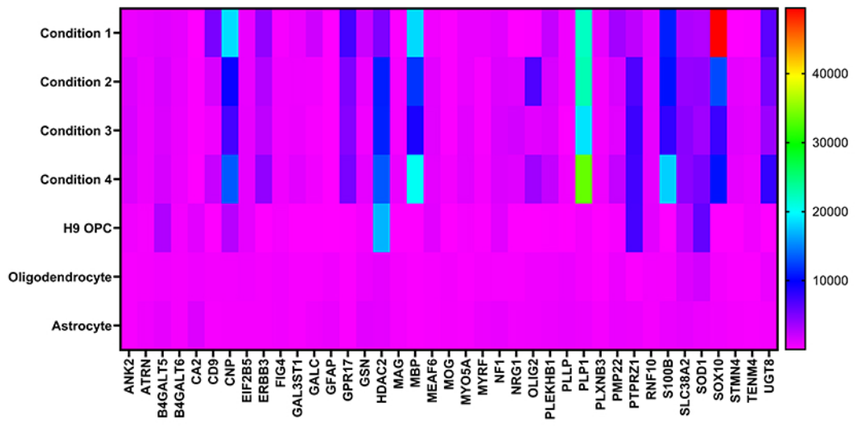


Figure 3. Gene Expression Profiles of Human Pluripotent Stem Cell Derived and Primary Human Glial Cells. The heatmap illustrates the expression levels of key oligodendrocyte-related genes across various experimental conditions, analyzed using NanoString technology. BrainXell's hiPSC-derived oligodendrocyte precursor cells (OPCs) were compared to human embryonic stem cell derived OPCs and primary oligodendrocytes and astrocytes isolated from human adult brain tissue. The expression profile of BrainXell's OPCs highlights numerous genes involved in oligodendrocyte differentiation, maturation, signaling, support, and myelination.

Functional Maturation of Oligodendrocytes

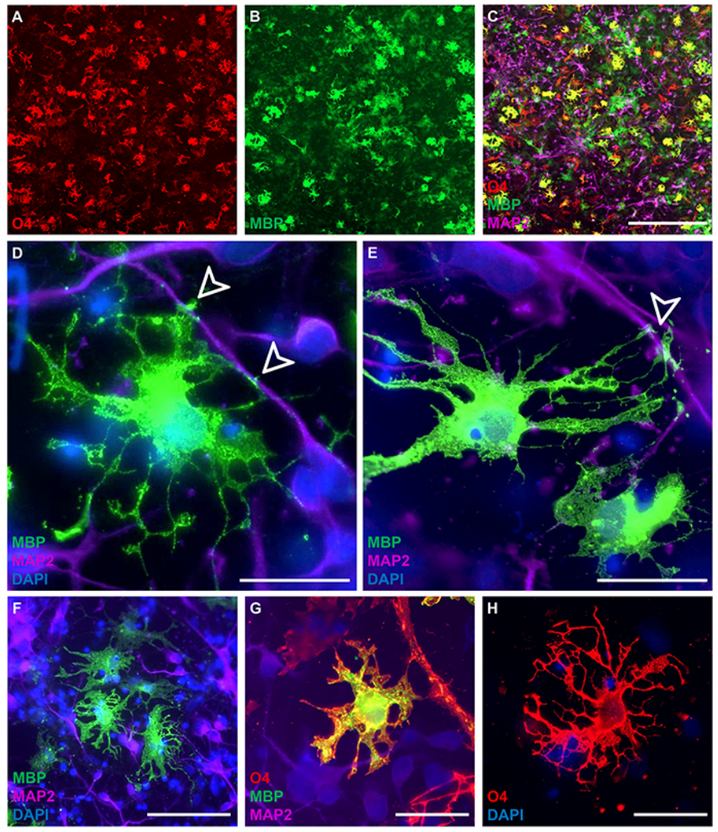


Figure 4. Arborized Morphology and Functional Maturation of Oligodendrocyte Progenitor Cells. (A-C) OPCs were co-cultured with cortical glutamatergic neurons for 14 days, with OPCs labeled for O4 (red) and MBP (green), and neurons labeled for MAP2 (far red). (D-E) MBP+ oligodendrocyte projections were observed wrapping around MAP2+ neurites (arrows), indicating functional interaction between the cell types. (F-G) Complex arborized morphology was evident in both co-cultures and monocultures (H), showcasing the maturation and structural development of OPCs in different environments. Scale bars = 500µm (C), 50µm (D-E), 100µm (F), 50µm (G-H).

Conclusion

- Culturing OPCs for 2 or 7 days under optimized conditions significantly increased MBP expression, with 60-80% of cells positive for MBP.
- Flow cytometry revealed ~80% of cells expressed the oligodendrocyte marker O4 in the best differentiation condition.
- qPCR analysis showed upregulation of oligodendrocyte-related genes across all conditions, indicating successful differentiation.
- NanoString analysis highlighted key genes involved in oligodendrocyte differentiation and maturation in BrainXell's hiPSC-derived OPCs compared to other sources.
- Co-culture with cortical neurons resulted in MBP+ projections wrapping around MAP2+ neurites, showcasing functional interactions.
- Complex arborized morphology of OPCs was evident in both co-cultures and monocultures, reflecting effective maturation.