Differentiating Human Pluripotent Stem Cells to Neurons: Approaches in Media Development

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ABSTRACT

Specialized cell culture media is a foundational tool for researchers working in diverse areas, from basic and applied research to biopharmaceutical applications. Thermo Fisher Scientific offers media systems for culture of human and rodent (primary) neural cell types and more recently has focused on identifying conditions that drive stem cell differentiation toward specific neural lineages. AIM: To develop new cell culture systems that enable robust differentiation of human pluripotent stem cells (PSCs) to distinct neuronal subtypes. METHODS: We have adopted a multifaceted approach for driving PSC to neuronal differentiation: 1. Disconnecting specification/regionalization studies from maturation and enabling parallel development activities. 2. Utilizing complex Design of Experiment (DOE) approaches and mathematical modeling paired with validated endpoint assays; 3. Incorporating small molecule chemical library screening to identify compounds with desired properties. RESULTS: We demonstrate the feasibility of distinguishing PSC specification from neuronal maturation by utilizing banks of neural stem cells (NSCs), produced in 7 days using Gibco® Neural Induction Medium. The NSCs provide a model to screen and optimize conditions driving neural differentiation and maturation. Additional results of definitive screening DOEs as well as modeling predictions are described. CONCLUSIONS: In the last several years significant advances in stem cell biology have enabled broader adoption of these cells and provided deeper insight into the mechanisms which regulate their growth and specific cell fate determination. In this work we present our approach to harness this insight to develop next generation culture systems to create useful neuronal cell models from PSCs.

INTRODUCTION

Figure 1. Development of central nervous system and approach to recapitulate it with in vitro system

RESULTS

Figure 2. Media development process

Differentiation of DA neurons: instead of staggered developmental approach, we used PSC as cell model to derive specific progenitor of midbrain floor plate cells and NSC as cell model to optimized maturation condition.

Figure 3. Example of Definitive Screening Design (DOE) to differentiate hPSC into FP/vmDPC

Figure 4. Development of maturation medium

Figure 5. Measurement system analysis / Assay development for Non Hypothesis driven screening study

Figure 6. Small molecule library screening

Figure 7. Bridging Specification and Maturation medium

CONCLUSIONS

REFERENCES

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