invitrogen

CRACKING THE DIFFERENTIATION POTENTIAL: BIOLOGICAL MODELS TO ADVANCE STEM CELL RESEARCH

Jamuna KS, Sreerag V, Suchitra Sajja and Sudha Balasubramanian
Thermo Fisher Scientific, Second Floor, First Technology Place, 3EPIP, Whitefield, Bengaluru, Karnataka 560066, India

ABSTRACT

The pluripotent nature of stem cells makes them a valuable resource for research and regenerative medicine. Induced pluripotent stem cells (iPSCs), neural stem cells (NSCs) and mesenchymal stem cells (MSCs) differentiated along various lineages can provide insights into proteins involved in disease or developmental processes, which would otherwise be inaccessible for research. The extensive application of these models from developmental biology to gene therapy generates a consistent need to efficiently characterize them. We have differentiated human iPSCs and NSCs to detect and characterize proteins present in their most relevant biological models: Alpha-actinin 2 and Troponin I in cardiomyocytes, insulin in the beta cells of the pancreas, and nestin, optineurin and GFAP in neural cells. We also developed 3D organoid models of intestine and kidney which have helped to develop and validate* antibodies against proteins like Sox9 and podocalyxin. Sox9 is an important transcription factor which regulates the proliferation and differentiation of mammalian intestinal epithelial stem cells. Thus, intestinal organoid was used as the most relevant biological model for Sox9 antibody validation. Similarly, since podocalyxin is expressed by the podocytes of kidney, which help in the formation and maintenance of podocyte foot processes, and is otherwise absent in undifferentiated iPSCs, the antibody against podocalyxin was validated using kidney organoid. We have also differentiated MSCs into adipocytes, osteocytes and chondrocytes in order to study the change in expression of proteins involved in the various stages of differentiation. For example, we studied the differential expression of the key transcription factor RUNX2 in the commitment of mesenchymal progenitors to osteoblast lineage. Given their importance and wide use in stem cell research, there is an absolute need for extensively tested and specific antibodies for key markers of pluripotency and differentiation. Our data demonstrate the utility of using antibodies to characterize stem cell progeny and conversely to use differentiated cells to validate antibodies in the proper cell model. Through this holistic understanding of the target protein biology, we have developed antibodies and validated their use to support stem cell research broadly.

INTRODUCTION

Stem cells are a population of undifferentiated cells characterized by their ability to extensively proliferate (self-renewal) and differentiate into different types of cells and tissues. These cells have expanded our understanding of developmental biology as well as the pathogenesis of diseases. Stem cells at various stages of differentiation are characterized by different proteins and identification of them is critical for their understanding in stem cell biology. At the same time, stem cells differentiated along various lineages can be a powerful tool for determining the cell and tissue specificity of antibodies. iPSCs are generated using a combination of four reprogramming factors, including Oct4, Sox2, Klf4 and c-Myc which retains the differentiation potential towards the ectodermal, endodermal, and mesodermal lineages. NSCs are an invaluable resource for neuroscience and neural stem cell studies. Additionally, NSC differentiations can be used to study the expression level of proteins in neurodegenerative conditions such as Parkinson's or Alzheimer's disease. Similarly, human mesenchymal stem cells (hMSCs) demonstrate the property of multipotency and can be differentiated into adipocytes, osteocytes and chondrocytes which can be identified by the expression of a unique combination of cell surface markers and transcription factors. Antibodies raised against proteins that are expressed in these specialized cells can be validated using this model system. Thus, stem cells and the differentiation models can help in overcoming the most significant challenge in antibody validation which is obtaining biologically relevant models especially when the protein of interest is of embryonic or rare tissue origin.

MATERIALS

Gibco™Human Episomal iPSC Line (A18945), Essential 8™ Medium (A1517001), Geltrex™ LDEV-Free, hESC-Qualified, Reduced Growth Factor Basement membrane (A1413301), RPMI 1640 (61870), B-27 supplement containing insulin (17504-044), Activin A Recombinant Human Protein (PHC9561), L-Glutamine100X(25030-081), FGF4 Recombinant Human Protein (PHG0154), FGF10 Recombinant Human Protein (A42546), Advanced DMEM-F12 (12634-010), HEPES buffer (15630080), Noggin Recombinant Human Protein (PHC1506), EGF Recombinant Human Protein Solution (PHG0311L), IGF1R Recombinant Human Protein (PR4654A), CMRL Medium (11530037), PSC Cardiomyocyte Differentiation Kit (A2921201), PSC Definitive Endoderm Induction Kit (A3062601), StemPro™ Neural Stem Cells (A15654), StemPro® Adipogenesis Differentiation Kit (A10070-01), StemPro® Osteogenesis Differentiation Kit (A10072-01), StemPro® Chondrogenesis Differentiation Kit (A10071-0) were procured from Gibco.

To learn more, visit : https://www.thermofisher.com/in/en/home/life-science/antibodies/primary-antibodies/research-area-antibodies/stem-cell-research-antibodies.html

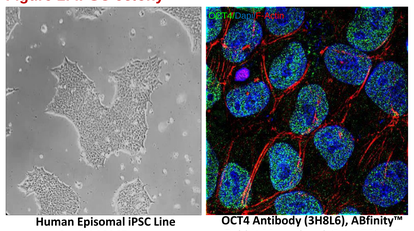
METHODS

Different protocols were followed to differentiate the stem cells. iPSC differentiation to Retinal Ganglion cells: Modified protocol of Gill, K. P. et al.(2016), iPSC differentiation to Schwann cell progenitor cells: Modified protocol of Kim, H.S. et al.(2017) iPSC differentiation to Neural Rosettes: Modified protocol of Kim, H.S. et al.(2017), iPSC differentiation to Intestinal organoid: Modified protocol of McCracken,K.W, et al. (2006), iPSC differentiation to Kidney organoid: Modified protocol of Morizane, R., et al (2017), iPSC differentiation to Hormone expressing pancreatic cells: Modified protocol of D'Amour, K. A et al. (2006). iPSC, NSC and MSC culture were performed according to the Gibco protocols. NSC differentiations to Neuron and Astrocytes were done according to Gibco® Neurobiology Protocols handbook . PSC Cardiomyocyte Differentiation Kit (modified) and PSC Definitive Endoderm Induction Kit protocols were used for cardiomyocyte and definitive endoderm differentiations.

* For Research Use Only. Not for use in diagnostic procedures.

RESULTS

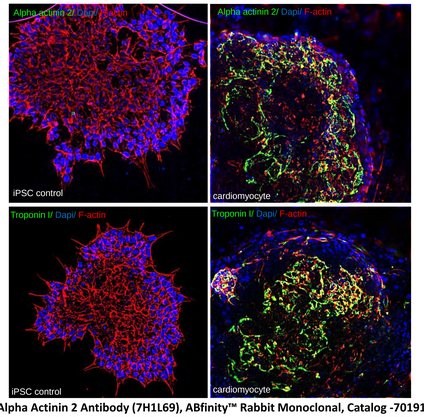
Figure 1: iPSC colony



Human Episomal iPSC Line Cat # A18945

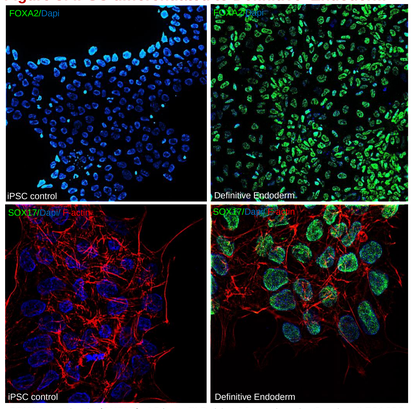
OCT4 Antibody (3H8L6), ABfinity™ Rabbit Monoclonal Cat # 701756

Figure 2: iPSC differentiated to Cardiomyocyte



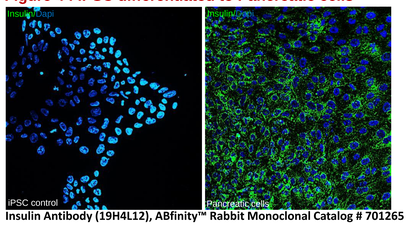
- Alpha Actinin 2 Antibody (7H1L69), ABfinity™ Rabbit Monoclonal, Catalog -701914
- Troponin I Antibody (1H11L19), ABfinity™ Rabbit Monoclonal, Catalog -701585

Figure 3: iPSC differentiated to Definitive Endoderm



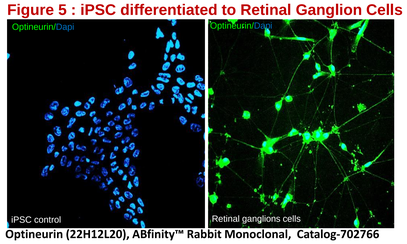
- FOXA2 Antibody (9H5L7), ABfinity™ Rabbit Monoclonal, Catalog # 701698
- SOX17 Antibody (6H42L1), ABfinity™ Rabbit Monoclonal, Catalog # 703063

Figure 4 : iPSC differentiated to Pancreatic cells



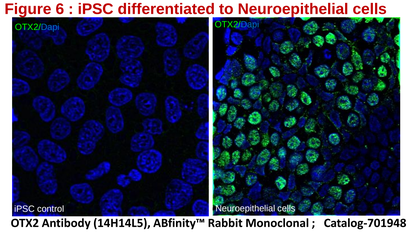
Insulin Antibody (19H4L12), ABfinity™ Rabbit Monoclonal Catalog # 701265

Figure 5 : iPSC differentiated to Retinal Ganglion Cells



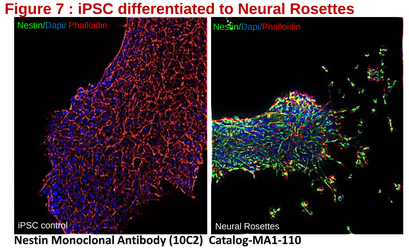
Optineurin (22H12L20), ABfinity™ Rabbit Monoclonal, Catalog-702766

Figure 6 : iPSC differentiated to Neuroepithelial cells



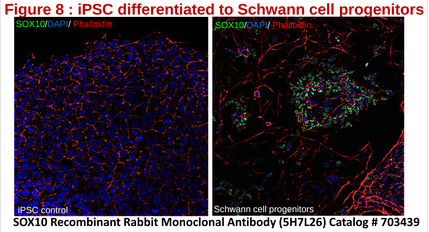
OTX2 Antibody (14H14L5), ABfinity™ Rabbit Monoclonal ; Catalog-701948

Figure 7 : iPSC differentiated to Neural Rosettes



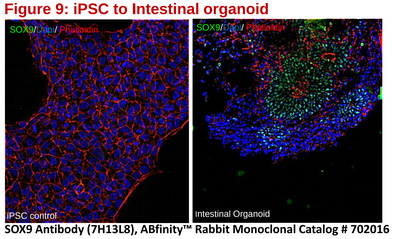
Nestin Monoclonal Antibody (10C2) Catalog-MA1-110

Figure 8 : iPSC differentiated to Schwann cell progenitors



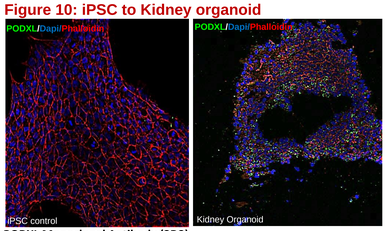
SOX10 Recombinant Rabbit Monoclonal Antibody (5H7L26) Catalog # 703439

Figure 9: iPSC to Intestinal organoid



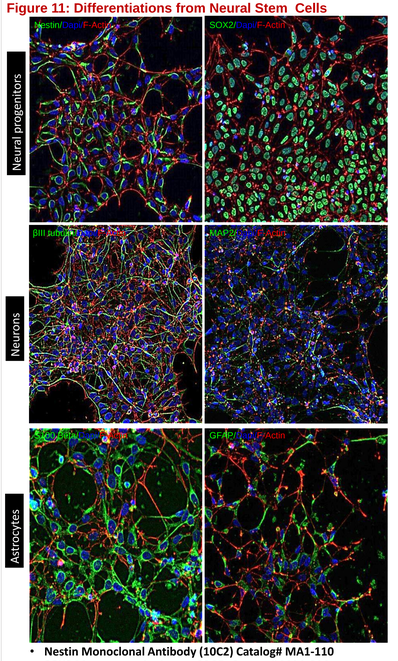
SOX9 Antibody (7H13L8), ABfinity™ Rabbit Monoclonal Catalog # 702016

Figure 10: iPSC to Kidney organoid



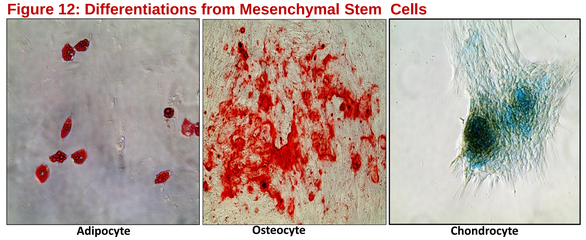
PODXL Monoclonal Antibody (3D3)

Figure 11: Differentiations from Neural Stem Cells



- Nestin Monoclonal Antibody (10C2) Catalog# MA1-110
- SOX2 Monoclonal Antibody (20G5) Catalog# MA1-014
- MAP2 Monoclonal Antibody (M13) Catalog# 13-1500
- β-III tubulin Monoclonal Antibody, Catalog# MA1-9587
- GFAP Monoclonal Antibody (ASTRO6) Catalog # MA5-12023

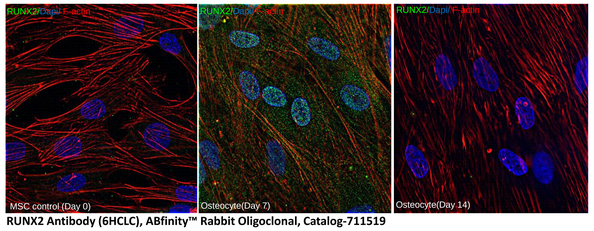
Figure 12: Differentiations from Mesenchymal Stem Cells



Adipocyte (Oil Red O staining)

Osteocyte (Alizarin Red staining)

Chondrocyte (Alcian Blue staining)



RUNX2 Antibody (6HCLC), ABfinity™ Rabbit Oligoclonal, Catalog-711519

DISCUSSION

Stem cells differentiated along various lineages can be used as powerful tool for developing and validating antibodies that demonstrate specificity through the recognition of specific biomarkers. Upon differentiation, many of the pluripotency genes get downregulated whereas the markers of differentiation get upregulated. For example, we have validated the specificity of Oct4 antibody using undifferentiated stem cells because Oct4 is a gatekeeper for ES cell pluripotency (Figure 1).The differentiated models are defined by a set of proteins and the identification of these key proteins become a critical step in stem cell research. Thus, we have characterized each cell type using specific antibodies like Alpha-actinin 2 and Troponin I in cardiomyocytes, insulin in the beta cells of the pancreas, optineurin in retinal ganglion cells, nestin in neural rosettes and SOX10 in schwann cell progenitors (Figure 2,4,5,7,8). Similarly, we generated intestine and kidney organoids which served as the most relevant biological models for the validation of SOX9 and PODXL antibodies (Figure 9,10). SOX9 is expressed in the epithelial stem cell zone of small intestine and PODXL plays an important role in maintenance of the intricate glomerular podocyte for optimal filtration in the kidney. We have also differentiated H9-derived human neural stem cells (NSCs) into neurons and astrocytes for the validation of beta-III-tubulin and GFAP antibodies (Figure 11). The loss of these proteins are indicators of neuronal degeneration and hence there is an immediate need to detect and further investigate for future studies. The stringent validation ensures that the antibodies are validated in the most relevant physiological model in addition to the generic cell lines. In few instances, the different stages of differentiation are characterized by the varying levels of specific proteins. This property is explored to validate and show the specificity of antibodies targeted towards proteins involved in different stages of differentiation. For example, we have differentiated multipotent mesenchymal stem cells (MSCs) into adipocytes, osteocytes and chondrocytes. We have used osteocytes to validate RUNX2 antibody; the expression of which increases by day 7 and decreases by day 14 (Figure 12). Thus, stem cells and the differentiations can be used as efficient model systems to demonstrate endogenous protein identification in the most specific cell model

REFERENCES

- Nat Biotechnol. 2006;24(11):1392-1401. doi:10.1038/nbt1259
- Scientific Reports, *2016* doi:10.1038/srep30552
- Nat Protoc 6, 1920–1928 (2011). https://doi.org/10.1038/nprot.2011.410
- Nat Protoc 12, 195–207 (2017). doi.org/10.1038/nprot.2016.170
- Stem Cell Reports. 2017;8(6):1714-1726. doi:10.1016/j.stemcr.2017.04.011

ACKNOWLEDGEMENTS

I would like to thank Dr David Piper and Dr Dinesh Palanivelu for all the motivation and guidance. I would also like to thank the entire cell culture team and the ICC team for their constant support and also Vanitha Lokesh for helping in the timely procurement of reagents.

ThermoFisher SCIENTIFIC