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Harnessing Mechanobiology of Human Pluripotent Stem Cells for Regenerative Medicine

Yubing Sun[†] and Jianping Fu*[,†,‡]

[†]Department of Mechanical Engineering and [‡]Department of Biomedical Engineering, University of Michigan, Ann Arbor, Michigan 48109, United States

ABSTRACT: Recent advances in human pluripotent stem cells (hPSCs) open new doors for therapeutics of motor neuron (MN)-associated neurodegenerative diseases. However, the MN differentiation process is not yet completely understood. In this Viewpoint, we stress the concept of designing synthetic cell culture surfaces with precisely controlled mechanical properties (such as rigidity) to improve the efficiency of MN differentiation from hPSCs. Emerging evidence strongly supports the potent role of mechanobiology in controlling stem cell fate. Leveraging the intrinsic mechanosensitive properties of hPSCs in conjunction with a synthetic elastomeric micropost array system, we have recently demonstrated significantly improved MN differentiation from hPSCs. Mechanotransduction mechanisms of hPSCs are an unexplored territory and likely involve coordination and cross-regulations of multiple targets and pathways including cell surface receptors, signaling transduction molecules, and nuclear proteins. We envision that research in hPSCs for MN degenerative diseases will benefit from accumulating knowledge of mechanobiology of hPSCs.

KEYWORDS: *Motor neuron, mechanobiology, rigidity, extracellular matrix, ALS*

Progressive degeneration and death of spinal cord motor neurons (MNs) are associated with fatal neurological diseases such as spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS). Our limited understanding of the complicated and diverse neuropathologies of these debilitating diseases has significantly hindered developments of effective strategies for clinical intervention and treatments. While mutation in survival of motor neuron-1 (SMN1) gene has been identified as responsible for the majority of patients afflicted with SMA, about 80% of ALS cases do not have a genetic origin. Several genes including SOD1, TDP43, and FIG4 are found to be associated with the remainder 20% of familial ALS cases, although a detailed mechanism of these mutations leading to ALS is still unclear. Moreover, the available animal models for studying ALS do not completely recreate the human disease. Consequently, none of the drugs tested effective in animal models has been proven effective for treating ALS patients.

Recent advances in human pluripotent stem cells (hPSCs) including human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) have opened exciting new doors for treating MN-associated neurodegenerative diseases. hPSCs have unlimited self-renewal capability in vitro, critical for generating enough cells for large-scale cell-based applications. Furthermore, hPSCs have the important potential to differentiate into various stages of progenitors with distinct epigenetic and transcriptional statuses, allowing selection of optimal cell types for stem cell-based transplantation therapies. Current stem cell-based clinical trials for treating MN-associated neurodegenerative diseases mainly use human adult stem cells including human spinal stem cells and human mesenchymal stem cells. Although adult stem cells can provide a supportive environment to remaining neurons in the diseased host, in many cases they fail to generate new functional neurons that can rescue neuronal circuit or form neuromuscular junctions. Moreover, hPSCs, especially patient-derived hiPSCs, may drastically change the way of drug discovery efforts by eliminating the genetic and anatomical differences between human and animals while offering exciting promise for personalized medicine and understanding patient-specific basis of disease.

All potential applications of hPSCs for treating MN-associated neurodegenerative diseases require large-scale manufacturing of clinical-grade neural stem cells and MNs. However, current approaches for deriving MNs are inefficient and time-consuming, presenting a significant technical hurdle for cell-based drug screening and transplantation applications. Most current approaches for in vitro derivation of MNs in some way mimic the process of neurogenesis during development (Figure 1a), requiring inhibition of BMP signaling for neuroepithelial fate induction before activation of caudalization and ventralization signals using morphogens such as fibroblast growth factors (FGFs), retinoic acid (RA), and sonic hedgehog (SHH). To recapitulate neuronal development, either recombinant proteins (such as noggin) or BMP receptor inhibitors (such as LDN193189) have been applied in hPSC culture to inhibit BMP signaling. Similarly, RA, SHH, and purmorphamine (Pur) have been employed for anterior/posterior and dorsal/ventral patterning of neural progenitor cells. However, BMP inhibitors are dispensable if embryonic bodies are used for neural differentiation of hPSCs, and RA-independent MN specification has also been reported recently. Therefore, our understanding of the regulatory network controlling MN differentiation remains incomplete.

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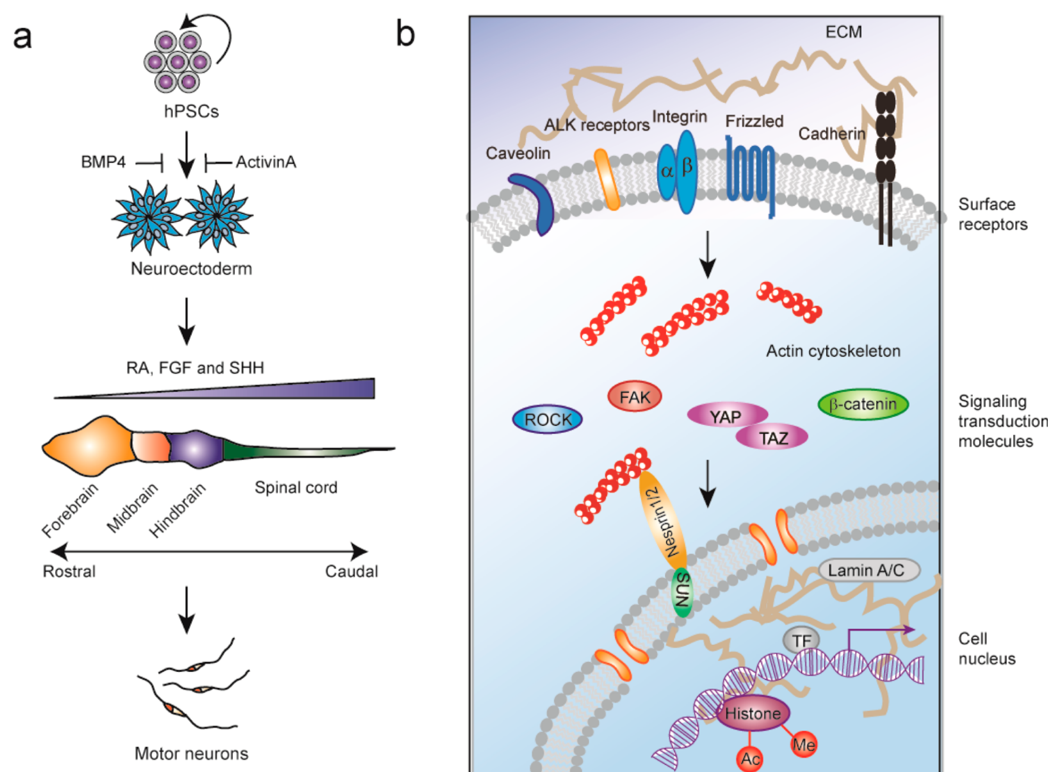

Figure 1. (a) MN specification follows developmental rules. After gastrulation, the inner cell mass differentiates into ectoderm, mesoderm, and endoderm. With signals inhibiting BMP4 and activin, cells in the dorsal region of the ectoderm are further developed into neuroepithelial cells. Morphogens such as RA, FGF, and SHH determine anterior/posterior and dorsal/ventral patterning. Spinal cord MNs possess simultaneously posterior and ventral identities. (b) Mechanotransduction as a multitargeted process. Cell surface receptors such as integrins, caveolin, ALK receptors, cadherins, and frizzled (Wnt receptors) are known to be mechanosensitive. Kinases such as Rho-associated protein kinase (ROCK) and focal adhesion kinase (FAK) and transcription factors such as YAP/TAZ and β-catenin can relay mechanical signals from the cell surface to the nucleus. Remodeling of the actin cytoskeleton can directly influence gene expression through nesprin, SUN proteins, and nuclear matrix protein Lamin A/C. Histone modification such as demethylation and deacetylation may also be regulated directly by biophysical cues in the cell microenvironment.

Over the past few years, there is a significant surge of interest in investigating the effect of the physical aspect of cell microenvironment on controlling stem cell fate. Indeed, mechanoresponsive behaviors of human adult stem cells including hematopoietic, mesenchymal, neural, and skeletal muscle stem cells have been well documented recently. Furthermore, evidence gathered from studies with mouse ESCs (mESCs) has supported the notion that, like many adult stem cells, ESCs may also be intrinsically mechanosensitive. For example, applying local cyclic stress using magnetic beads to mESCs via integrin-mediated focal adhesions can result in loss of pluripotent markers.[1] These important findings have shed light on the importance of biophysical cues in the local cell microenvironment (i.e., rigidity and mechanical force) during embryonic development and for improving in vitro culture of ESCs.

To investigate specifically the instructive role of substrate rigidity on hPSC fate regulation, we and others have recently utilized different biomaterial systems with precisely controlled mechanical properties to regulate hPSC−biomaterial interactions.[2] Specifically, we have developed a synthetic, micromolded poly(dimethylsiloxane) (PDMS) micropost array (PMA) system, which has a uniform surface geometry and different post heights to modulate substrate rigidity independent of effects on adhesion and other material surface properties. Using the PMA, we have very recently demonstrated that substrate rigidity is an in vitro extracellular switch that directs lineage decisions of hPSCs between neuroepithelial cells (NEs) and neural crest cells (NCs). In a chemically defined culture condition supportive of neuroectoderm differentiation, soft PMAs (with a substrate rigidity $K < 5$ kPa) promote NE conversion of hPSCs. More importantly, even without patterning cues such as RA, soft PMAs (with $K < 5$ kPa) can significantly enhance expression of several HOX genes, which are essential for the anterior/posterior axis patterning, to induce caudalized NEs and $Olig2^+$ MN progenitor cells. The purity and yield of functional MNs derived from hPSCs within 23 days of culture using soft PMAs are improved more than 4-fold and 10-fold, respectively, compared with coverslips or rigid PMAs. We have further used whole-cell recording to confirm that MNs derived from soft PMAs exhibit electrophysiological activities comparable to those from primary neurons in vivo. In distinct comparison, hPSCs require up to 50 days under conventional differentiation conditions for complete functional maturation into a MN.

Our result showing accelerated neural induction of hPSCs on soft cell culture surfaces is consistent to a previous study where Matrigel coated polyacrylamide (PA) gels with different bulk rigidities were used for examining mechanosensitive properties of hPSCs.[2b] In another recent study, PA gels functionalized with an adhesive peptide (GKKQRFRHRNRKG) derived from vitronectin were utilized for examining the effect of substrate rigidity on stemness maintenance of hPSC.[2c] In this work, the authors have shown that only rigid PA gels functionalized with

the adhesive peptide can maintain hPSC proliferation and pluripotency, which is consistent with one of our earlier studies where we have shown that rigid PMAs coated with vitronectin support maintenance of pluripotency of hPSCs. All together, our studies in conjunction with others have provided convincing evidence for the intrinsic mechanosensitive properties of hPSCs.

Detailed molecular mechanisms responsible for mechanosensitive behaviors of hPSCs are still elusive, and likely mechanotransduction of hPSCs requires cellular responses and regulations at multiple levels (genetic and epigenetic, transcriptional, and post-transcriptional including microRNA) and time scales (Figure 1b). It has been demonstrated that cell surface receptors and adhesion molecules (e.g., integrins), intracellular signaling molecules (e.g., YAP and RhoA/ROCK), nuclear components (e.g., SUN proteins and lamin A/C), and transcriptional factors (e.g., NF-κB and EGR1) all contribute to mechanosensitivity and -responsibility of mammalian cells. Our mechanistic study also reveals a multitargeted mechanotransductive process in hPSCs, in which soft substrates can simultaneously inhibit Smad 1/5/8 phosphorylation, lead to disassembly of actin microfilaments, and activate kinase Lats which in turn phosphorylates YAP to prevent nuclear translocation of phosphoSmads and inhibit subsequent activation of Smad target genes.[2a] It is also worth noting that, in a recent study by Downing et al., the authors have discovered that patterned cell culture surfaces can lead to increased histone H3 acetylation and methylation by reducing histone deacetylase activity and upregulation of the expression of WD repeat domain 5 (WDR5), a subunit of H3 methyltranferase in fibroblasts during cell reprogramming, highlighting the effect of biophysical cues on directly modifying epigenetic status.[3] It is important to recognize mechanotransduction as an integrated cellular regulation process that can effectively drive hPSCs toward specific phenotypes with associated functions.

Mechanobiology of hPSCs is still in its infancy; there are many important questions unanswered that require concerted and collaborative efforts from stem cell biologists and biomaterial scientists. We envision that mechanobiology of hPSCs will be under intense study in the coming years given its importance in development and for rational designs of synthetic culture systems for large-scale manufacturing of hPSCs and their functional derivatives (such as MNs for MN-related neurodegenerative diseases). Future studies will likely focus on identifying the role of dynamic biophysical cues on differentiation of hPSCs toward other lineages and subpopulations of progenitors and stem cells. Another critical research direction is to tease out detailed signaling pathways and regulatory networks that are responsible for relying biophysical signals from extracellular space to regulate gene expressions and fate control of hPSCs.

AUTHOR INFORMATION

Corresponding Author

*E-mail: jpfu@umich.edu.

Notes

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