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Characterization and neural differentiation of mouse embryonic and induced pluripotent stem cells on cadherin-based substrata

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ABSTRACT

A suitable culture condition using advanced biomaterials has the potential to improve stem cell differentiation into selective lineages. In this study, we evaluated the effects of recombinant extracellular matrix (ECM) components on the mouse embryonic stem (mES) and induced pluripotent stem (miPS) cells' self-renewal and differentiation into neural progenitors, comparing conventional culture substrata. The recombinant ECMs were established by immobilizing two chimera proteins of cadherin molecules, E-cadherin-Fc and N-cadherin-Fc, either alone or in combination. We report that the completely homogeneous population of mES and miPS cells could be maintained on E-cadherin-based substrata under feeder- and serum-free culture conditions to initiate neural differentiation. Using defined monolayer differentiation conditions on E-cadherin and N-cadherin (E-/N-cad-Fc) hybrid substratum, we routinely obtained highly homogeneous population of primitive ectoderm and neural progenitor cells. Moreover, the differentiated cells with higher expression of βIII-tubulin, Pax6, and tyrosine hydroxylase (TH) in absence of GFAP (a glial cell marker) expression suggesting the presence of a lineage restricted to neural cells. Our improved culture method should provide a homogeneous microenvironment for differentiation and obviate the need for protocols based on stromal feeders or embryoid bodies.

1. Introduction

The embryonic stem (ES) cells and induced pluripotent stem (iPS) cells represent a promising source to overcome many human diseases by providing an unlimited supply of differentiated cells, including cells with neural characteristics [1–3]. During embryogenesis in vivo, the neurons arise from the neuroectodermal precursors [4]. Efficient production of these ectoderm progenitors would allow on-demand production of neurons with different subtypes [5,6].

Efforts have been devoted to producing defined lineages of neural cells from ES and iPS cells. Most of the protocols for neural differentiation of ES cells relied on the formation of cluster of cells, so-called embryoid bodies (EBs) [7,8] or neurospheres [9], to initiate differentiation. Although initial studies on the generation of defined lineages of neural cells seemed encouraging, later work revealed that the neuronal clusters derived from ES and iPS cells contain a variety of neuronal subtypes as well as non-neuronal lineages, including undifferentiated cells [7,10]. Since then, progress has been made to enrich neural subtypes with special emphasis on inductive signals [11,12], transcription factors [6], and cell adhesion molecules (CAMs) for extracellular matrix [7,13]. Among them, cell adhesion molecules could act as obvious candidates for guiding differentiation into uniform and defined lineages.

The regulation of stem cell behavior and the formation of appropriate neural circuits depend on a complex interplay between extracellular guiding cues and intracellular signaling [14]. Two members of cadherin superfamily, E-cadherin and N-cadherin, are most-studied CAMs involved in the ES cell pluripotency and neural development, respectively [15,16]. Recent findings showed the important effect of E-cadherin to maintain pluripotency [17], reduce cellular heterogeneity [18,19], and improve iPS cell generation [20]. Moreover, N-cadherin acts as an important regulator of nervous system development by providing important molecular cues in many biological processes such as retina development, somite formation, and neurite outgrowth [15,21]. Both of these cadherins are differentially expressed, depending on developmental stage and cell type. Mouse (m) ES and iPS cells express high level of E-cadherin, which can act as pluripotent marker [17]. On the other hand, the neural differentiation of mES cells is associated

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