Characterization of human neural stem/progenitor cells and their application to assays for multiple ionotropic glutamate receptors

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Introduction

Ionotropic glutamate receptors (iGluRs) are well-known targets contributing to the central nervous system-related phenomena, including synaptic plasticity and excitotoxicity. To predict neuronal toxicity and therapeutic efficacy of drug candidates which act on these receptors, physiologically relevant assay system of human neural cells is necessary. We here utilized HIP-009 cells, which are human fetal hippocampus-derived neural stem/progenitor cells. In the present study, we characterized neural differentiation potential of HIP-009 cells and investigated their function of ionotropic glutamate receptors.

Experimental methods

Cell culture

HIP-009 cells were purchased from PhoenixSongs Biologicals (Branford, CT). Cells were expanded and differentiated as described in the manufacture's instructions. Briefly, cells were proliferated on laminin-coated dishes in Neural StemCell Growth Medium (PhoenixSongs Biologicals). Before the start of differentiation, expanded cells were plated on laminin-coated plates in Neural Transition Medium (PhoenixSongs Biologicals) for three days. After that, cells were seeded on poly-D-lysine-coated plates in Neural Differentiation Medium (PhoenixSongs Biologicals) for differentiation. The differentiation process was carried out for ~ 28 days, during which half of the media was changed twice a week.

Summary and conclusion

- HIP-009 cells under undifferentiated state expressed neural stem/progenitor markers.
- HIP-009 cells were differentiated into mixed population of neurons and astrocytes for ~ 28 days.
- Differentiated HIP-009 cells expressed ionotropic glutamate receptors functionally.
- AMPA-evoked Ca^{2+} influx was observed without desensitization inhibitor, CTZ.
- Agonists and antagonists of each ionotropic glutamate receptor were detected in the Ca^{2+} influx assay using HIP-009 cells.

	Agonists/EC_{50} (μM)	Antagonists/IC_{50} (μM)	Others
NMDARs	NMDA/7.47 ± 0.41	MK-801/0.63 ± 0.13 Memantine/6.73 ± 1.04 D-AP5/11.38 ± 1.82 7-CKA/1.07 ± 0.18	*Co-agonists/EC_{50} (μM)* Glycine/2.13 ± 0.24 D-serine/2.66 ± 0.25
AMPARs	AMPA/3.47 ± 0.33	NBQX/0.73 ± 0.06 CNQX/3.81 ± 0.57	*Desensitization inhibitor* CTZ potentiated total Ca^{2+} influx without change in sensitivity to AMPA stimulation
KARs	KA/33.47±1.14	NBQX/0.66 ± 0.03 CNQX/3.12 ± 0.40	———

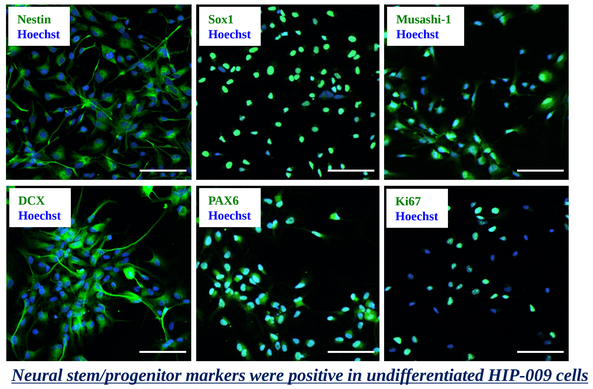
These observations indicate that HIP-009 cells are a novel physiologically relevant tool to evaluate effects of drug candidates on ionotropic glutamate receptors *in vitro*.

Abbreviations

NMDARs: *N*-methyl-D-aspartate receptors
AMPARs: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptors
KARs: kainic acid receptors
D-AP5: D-(-)-2-amino-5-phosphonopentanoic acid
7-CKA: 7-chlorokynurenic acid, glycine-binding site antagonist of NMDARs
CTZ: cyclothiazide, desensitization inhibitor of AMPARs

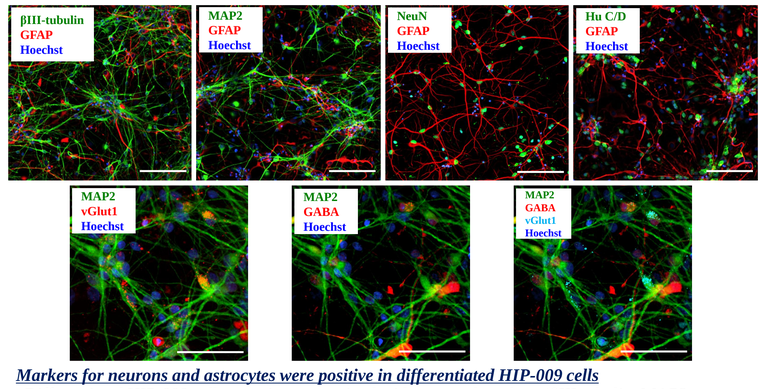
Results

Immunocytochemistry of neural markers



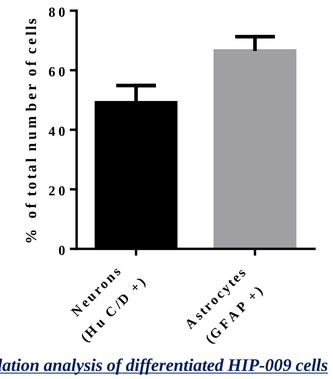
Neural stem/progenitor markers were positive in undifferentiated HIP-009 cells

Undifferentiated HIP-009 cells were immunostained with neural stem/progenitor markers, nestin, SOX1, Musashi-1, DCX and PAX6, and a proliferation marker, Ki67. Scale bar, 100 μm



Markers for neurons and astrocytes were positive in differentiated HIP-009 cells

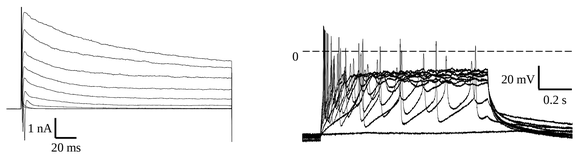
Differentiated HIP-009 cells were immunostained with neuronal markers (βIII-tubulin, MAP2, NeuN and Hu C/D), a glutamatergic neuron marker (vGlut1), a GABAergic neuron marker (GABA) and an astrocyte marker (GFAP). Scale bar, 100 μm (upper) and 50 μm (lower).



Population analysis of differentiated HIP-009 cells

A ratio of neurons (Hu C/D-positive cells) and astrocytes (GFAP-positive cells) among total cells was calculated. Values are expressed as means ± S.E.M. N = 10.

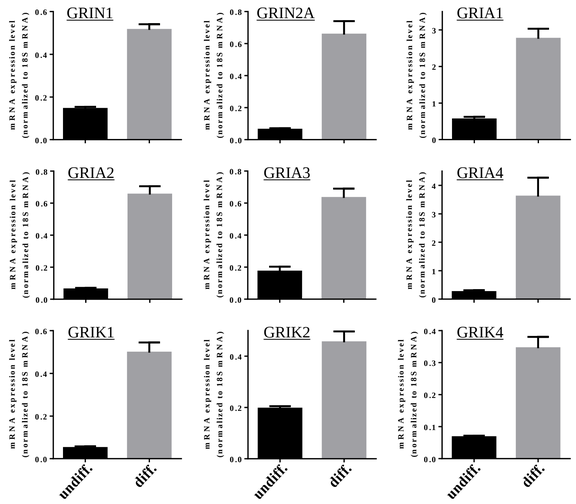
Electrophysiological functions



Electrophysiological recordings from differentiated HIP-009 cell

Representative traces of whole-cell voltage clamp (left) and current clamp (right).

mRNA expression of iGluRs

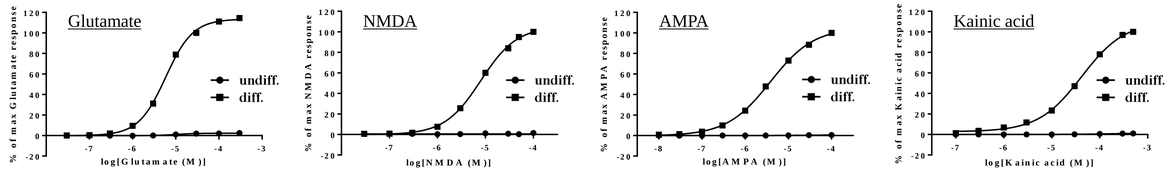


RT-qPCR analysis of HIP-009 cells

mRNA expression of NMDARs (GRIN1 and GRIN2A), AMPARs (GRIA1-4) and KARs (GRIK1, 2 and 4) was up-regulated in differentiated HIP-009 cells. undiff., undifferentiated HIP-009 cells; diff., differentiated HIP-009 cells. Values are expressed as means ± S.E.M. N = 4.

Ca^{2+} influx assay of iGluRs

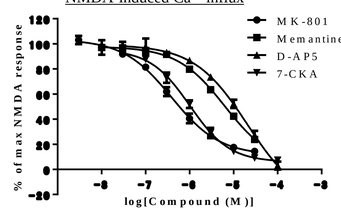
Agonists of each iGluR



Ca^{2+} influx in undifferentiated and differentiated HIP-009 cells by each agonist

EC_{50} values of each agonist were as follows: glutamate, 4.81 ± 0.30 μM; NMDA, 7.47 ± 0.41 μM; AMPA, 3.47 ± 0.33 μM; KA, 33.47 ± 1.14 μM. Values are expressed as means ± S.E.M. N = 3–4.

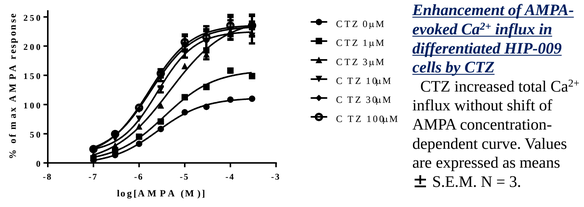
Antagonists of NMDARs



Inhibition of NMDA-evoked Ca^{2+} influx in differentiated HIP-009 cells

IC_{50} values of each antagonist were as follows: MK-801, 0.63 ± 0.13 μM; memantine, 6.73 ± 1.04 μM; D-AP5, 11.38 ± 1.82 μM; 7-CKA, 1.07 ± 0.18 μM. Values are expressed as means ± S.E.M. N = 3–4.

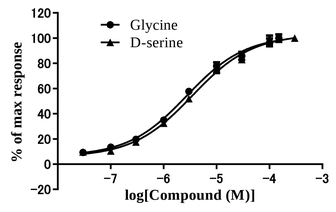
Desensitization inhibitor of AMPARs



Enhancement of AMPA-evoked Ca^{2+} influx in differentiated HIP-009 cells by CTZ

CTZ increased total Ca^{2+} influx without shift of AMPA concentration-dependent curve. Values are expressed as means ± S.E.M. N = 3.

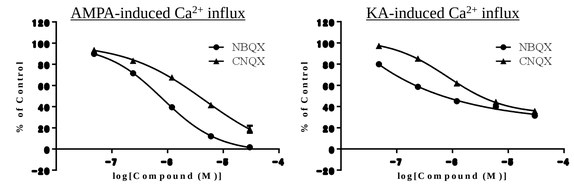
Co-agonists of NMDARs



Competitive assay of NMDAR-glycine site agonist and antagonist

NMDA-induced Ca^{2+} influx blocked by 7-CKA was recovered by glycine or D-serine. EC_{50} = 2.13 ± 0.24 μM and 2.66 ± 0.25 μM for glycine and D-serine, respectively. Values are expressed as means ± S.E.M. N = 3.

Antagonists of AMPARs/KARs



Inhibition of AMPA- or KA-induced Ca^{2+} influx

IC_{50} values of each antagonist were as follows: NBQX, 0.73 ± 0.06 μM and 0.66 ± 0.03 μM for AMPA-and KA-stimulation, respectively; CNQX, 3.81 ± 0.57 μM and 3.12 ± 0.40 μM for AMPA-and KA-stimulation, respectively. Values are expressed as means ± S.E.M. N = 3.