The spatial and temporal expression of MERG1 channels in undifferentiated and differentiated mouse neural stem cells

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ABSTRACT

The human ether-a-go-go related gene (hERG) encodes the rapidly activating delayed rectifier potassium channel. When this channel’s ability to conduct electrical current across the membrane is inhibited, it is known to cause long QT syndrome which is a fatal disorder. HERG’s involvement in cancer cell proliferation spurred this investigation of its spatial and temporal expression in neural stem cells (NSCs). Mouse NSCs were grown and differentiated on coated surfaces and their total RNA and proteins were extracted for analysis using RT-PCR and western blotting. Then, immunostained cells were visualized using confocal microscopy. Mouse ERG1 (mERG1) was observed in the cytoplasm and membrane compartment of NSCs and differentiated neural phenotypes throughout 14 days of differentiation. The transcriptional activity of mERG1 gradually increased. However, the western blot intensity of mERG1 proteins reduced as the cells differentiated. This leads to more questions on why the cells produce more mRNA than required which can be addressed in further studies.

INTRODUCTION

Neural stem cells (NSCs) are of great interest because the cells are multipotent, hence, retaining the ability to differentiate into neurons and glia. As such, they are an intriguing source of tissue for the study of cell proliferation and differentiation. Much research has been done on potassium channels and they were found to be involved in tumor proliferation (Wonderlin and Strobl, 1996). However, their role in cell proliferation is poorly understood. hERG belongs to the ether-a-go-go (EAG) family which is an evolutionary conserved multigenic family of voltage-activated K+ channels that which has three subfamilies: EAG, ELK and ERG (Littleton and Ganetzky, 2000). hERG’s importance in the cardiac myocytes had called for extensive research on its role in the heart but little has been done on the expression of hERG in neural stem cells (NSCs). Three different genes constituted the mammalian hERG subfamily: hERG1, hERG2 and hERG3 (Ishii \textit{et al}, 1997). The latter two are found only in the nervous system. This study seeks to find out more on the expression of hERG1 in undifferentiated and differentiated NSCs such as astrocytes,

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oligodendrocytes and neurons. Investigating more on the expression and location of mERG1 can allow further research done on the functions of hERG at its various locations. Understanding hERG functions in neural stem cells will be another big leap towards shedding light on its complex mechanism with regards to NSC proliferation and differentiation. Scientists can develop new drugs or therapies for neuronal associated disorders and channelopathies.

MATERIALS AND METHODS

NSCs from the hippocampus and subventricular zone (SVZ) of fetal mouse brain are extracted and after dissociating and removing the cell debris, the neural stem cells were cultured in 6 well dishes and grown in neurosphere medium containing DMEM/F12 (Gibco, CA,USA) supplemented with N2 supplement (Gibco), 20ng/ml Epidermal Growth Factor (EGF; Invitrogen, CA, USA), 20ng/ml basic Fibroblast Growth Factor (bFGF; Invitrogen), Penicillin (Sigma-Aldrich, MO, USA) and Streptomycin (Sigma-Aldrich). Passage three neurospheres were dissociated and counted. Then they were plated on individual wells of 24 well dishes, 6 well dishes and 100mm Petri dishes coated with PLO and FN to grow the cells in a monolayer. The samples were for immunostaining, RNA and protein collection, respectively. After growing for three days, the cells were induced to differentiate and the samples were collected at day 0, 1, 3, 5, 7, 14 post-differentiation.

RNA extraction and qPCR

PureLink™ Micro-Midi Total RNA purification system (Invitrogen) was used to extract and purify total RNA from the cells and then the concentration of RNA was quantitated using QuantiT RNA Assay kit (Invitrogen) according to manufacturer’s instruction. Reverse transcription was conducted using the master mix (Promega, Madison, WI, USA). The reaction was run in a Real Time PCR machine (ABI7500, Applied Biosystems).

Protein extraction and western blotting

Protein was isolated from the cells and quantitated using BCA Protein Assay kit (Pierce Chemicals) according to manufacturer’s instruction. 20µg of hippocampus or SVZ NSCs protein samples were mixed with 5x loading dye and heated. The samples were run through 4-15% pre-cast denaturing polyacrylamide gel (Biorad) at 100V for 2½ hours. After successful transfer to the nitrocellulose membrane, membrane was cut depending on the predicted molecular weight of ERG1 and beta-actin for protein detection during western blot. The membrane was blocked with 5% skim milk in 0.1% TBS-T and then incubated with primary antibody hERG (1:700; Santa Cruz) and beta-actin (1:10000) separately in 1% skim milk overnight at 4°C on an orbital shaker. After washing, they were incubated with secondary Goat anti-rabbit HRP (Pierce Chemicals) antibody (1:50000) in 1% skim milk. The blot was then incubated with West Femto Chemiluminescent substrate (Pierce Chemicals) and the signals were detected using an X-ray film via autoradiography.

Immunocytochemistry

The fixed cells were permeabilized with PBS+0.1%TX. and double immunostained with hERG primary antibody (1:150; Santa Cruz) in 3 %BSA overnight and with one of these second primary
antibodies: CNPase (1:200; MAB328R, Chemicon, CA, USA), GFAP (1:2000; Z0332, Dako, Denmark) or Tuj1 (1:500; MMS435P, Covance) for 2 hours. After every incubation of the primary antibody is followed by the secondary antibody: Alexafluor donkey anti-goat 555 IgG secondary antibody (1:200; Invitrogen) for anti-hERG1 and Alexafluor goat anti-rabbit 488 IgG or goat anti-mouse 488 IgG secondary antibody (1:200; Invitrogen) for the second primary antibody. The slides were imaged using Zeiss Laser Confocal Microscope (LSM 510, Carl Zeiss Microimaging GmbH, Germany).

Another set of triple immunostaining was carried using different second primary antibodies: Sox2 (1:1000; AB5603, Chemicon, CA, USA) and Vimentin (1:200; MAB1681, Chemicon). The secondary antibodies used for detection were Alexafluor goat anti-mouse 633 IgG2a and goat anti-mouse 488 IgM antibody (Invitrogen) at 1:200 dilution.

RESULTS

Figure 1 Western blot analysis of mERG1 channel protein extracted from (a) hippocampus and (b) SVZ NSCs.

![Western blot analysis of mERG1 channel protein extracted from (a) hippocampus and (b) SVZ NSCs.](image-url)
CONCLUSION

Expression of mERG1 in NSCs
MERG1 expression is present near the nucleus and also at the cytoplasm and plasma membrane where it functions as an active potassium channel. Transcriptional activity of mERG1 is the lowest compared to when the cells are differentiating.

Expression of mERG1 in differentiated NSCs
When the cells undergo differentiation for a period of 14 days, the intensity of glycosylated mERG1 channel proteins present at the plasma membrane reduces while unglycosylated mERG1 at the cytoplasm remains distinct. However, while the total mERG1 protein reduces, the transcriptional activity of mERG1 has a gradual increase.

REFERENCES


Littleton J.T., Ganetzky B. Ion channels and synaptic organization: analysis of the drosophila genome. Neuron 26 35-43 2000