Neuronal Differentiation of Spiral Ganglia Cells with BMP-4
Sarmela Sunder MD*, Kazuo Oshima MD, PhD1,2, Pascal Senn MD1,2 and Stefan Heller PhD1,2
1Stanford University Medical Center and School of Medicine, Department of Otolaryngology-Head & Neck Surgery, 2 Department of Molecular & Cellular Physiology

Abstract

Stem cells cultured from murine inner ear proliferate in vitro forming spheres, floating clonal colonies. Recent studies demonstrate the capability for sphere formation by cochlear cells. Certain factors induce differentiation of these cells. Bone morphogenetic protein-4 (BMP-4) is involved in signaling pathways of auditory system but has not been shown to promote neuronal differentiation of spiral ganglia (SG) cells. 

SGs of newborn Math-1/nuclear green fluorescence protein (nGFP) mice were dissected, then treated with trypsin and triturated. The cell suspension was used for sphere formation in culture. Media contained without BMP-4 and experimental wells contained media in concentrations of 0.3, 1, 3, 5, 10, 30 and 100 ng/ml. After 14-day cultures, cells were stained with neuronal markers followed by quantification of the presence of neurons. SG cells cultured with BMP-4 exhibited neuronal differentiation, and did so in a dose-response manner. All samples of SG spheres cultured in BMP-4, except for 100 ng/ml, resulted in the presence of neurons in quantities statistically significantly greater than the control samples. The highest concentration of BMP-4 (100 ng/ml) showed virtually no neurons, demonstrating an adverse effect of BMP-4 at high concentrations.

SGS were cultured in various concentrations of BMP-4 demonstrated neuronal differentiation in vitro. Concentrations of 5-30 ng/ml resulted in the greatest number of neurons derived from stem cells. This could be an efficient strategy for pre-treatment of neural progenitors used in transplantation studies to replace lost auditory neurons.

Introduction

More than 250 million people around the world suffer from hearing impairment. The role of inner ear derived stem cells as a potential source of replacement cells for sensory hair cell and auditory neuron degeneration, which are the leading causes of hearing impairment, has been introduced in the recent literature [1-3]. Stem cells from the murine inner ear proliferate in vitro forming spheres, floating clonal colonies. These spheres which have the capacity to self-renew and differentiate into various cell types have been isolated from the cochlea and vestibular apparatus of guinea pig, mice and humans. Recent studies demonstrate the capability for sphere formation by spiral ganglia (SG) cells [4-11]. Certain factors induce differentiation of these cells. Bone morphogenetic protein-4 (BMP-4) is involved in signaling pathways of auditory system but has not been shown to promote neuronal differentiation of spiral ganglia (SG) cells to date.

Methods and Materials

Isolation of spiral ganglion stem cells for sphere formation

To study cell differentiation, 20 equally sized second-generation spheres were transferred per experimental data point into plastic 6-well tissue culture plates (Brewer 35mm/10mm 4-well tissue culture dishes) coated with 0.1% gelatin (Chemicon-Millipore, MW 30000). spheres were added directly into the medium containing of DMEM/high-glucose and F12 media (mixed 1:1) supplemented with N2 and B27 supplements, EGF (20 ng/ml), bFGF (10 ng/ml), IGF-1 (50 ng/ml), and heparan sulphate (50 ng/ml). Generation of a single cell suspension was ensured through microscopical inspection. The suspension was then incubated at 37 °C for 7 days to obtain second-generation spheres. (See figure 1.)

Cell differentiation

To study cell differentiation, 20 equally sized second-generation spheres were transferred per experimental data point into plastic 6-well tissue culture plates (Brewer 35mm/10mm 4-well tissue culture dishes) coated with 0.1% gelatin (Chemicon-Millipore, MW 30000). spheres were added directly into the medium containing of DMEM/high-glucose and F12 media (mixed 1:1) supplemented with N2 and B27 supplements, EGF (20 ng/ml), bFGF (10 ng/ml), IGF-1 (50 ng/ml), and heparan sulphate (50 ng/ml). Generation of a single cell suspension was ensured through microscopical inspection. The suspension was then incubated at 37 °C for 7 days to obtain second-generation spheres. (See figure 1.)

Figure 1

Spiral ganglia spheres cultured in various conditions of BMP-4 demonstrated neuronal differentiation in vitro. Concentrations of 5-30 ng/ml resulted in the greatest number of neurons. BMP-4 treatment may be useful to increase the number of neurons derived from stem cells. This could be an efficient strategy for pre-treatment of neural progenitors used in transplantation studies to replace lost auditory neurons.

Results

Methods

1. R Martinez-Monedero, K Oshima, S Heller, AS Edge: BMP-4 treatment may be useful to increase the number of neurons derived from stem cells.

2. H Li, H Liu, S Heller: Concentrations of 5-30 ng/ml resulted in the greatest number of neurons derived from stem cells.

3. S Heller: Concentrations of 5-30 ng/ml resulted in the greatest number of neurons derived from stem cells.


7. S Heller: BMP-4 treatment leads to an increase of TuJ positive cells (i.e. neurons). Scale bar = 200 μm.

Figure 2

Figure 2

Figure 3

Figure 3

Conclusions

More than 250 million people around the world suffer from hearing impairment. The role of inner ear derived stem cells as a potential source of replacement cells for sensory hair cell and auditory neuron degeneration, which are the leading causes of hearing impairment, has been introduced in the recent literature [1-3]. Stem cells from the murine inner ear proliferate in vitro forming spheres, floating clonal colonies. These spheres which have the capacity to self-renew and differentiate into various cell types have been isolated from the cochlea and vestibular apparatus of guinea pig, mice and humans. Recent studies demonstrate the capability for sphere formation by spiral ganglia (SG) cells [4-11]. Certain factors induce differentiation of these cells. Bone morphogenetic protein-4 (BMP-4) is involved in signaling pathways of auditory system but has not been shown to promote neuronal differentiation of spiral ganglia (SG) cells to date.

Conclusions

The total number of cells (neuronal + non-neuronal cells) were similar throughout all experiments.

Figure 3

References


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