

An *In vitro* Study on Chick Somite Ability to Express Cerberus, Chordin, FGF8, Follistatin, and Noggin Transcripts

Samaneh Sadat Hosseini Farahabadi¹, Khadijeh Karbalaie², Hossein Salehi³, Farzaneh Rabiee², Kamran Ghaedi^{1,2†}, and Mohammad-Hossein Nasr-Esfahani^{2†}

1. Cell and Molecular Biology Division, Department of Biology, School of Sciences, University of Isfahan, Isfahan, Iran

2. Department of Cellular Biotechnology at Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran

3. Department of Anatomy, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

† These authors equally contributed to this work

Abstract

Background: *In vitro* simulation of developmental processes is an invaluable tool to shed light on the intrinsic mechanism of developmental biosystems such as central nervous system in mammals. Chick somites have been used to simulate the neural differentiation of human neural progenitor cells. In the present study, we aimed to indicate whether somites have the ability to express required neural differentiation factors at mRNA level.

Methods: Chick embryos were isolated from the yolk surface of the fertilized eggs and somites were subsequently isolated from embryos under a dissecting microscope. Total RNA of the somites was extracted and RT-PCR carried out with specific primers of cerberus, chordin, FGF8, follistatin and noggin.

Results: Data showed that five aforementioned factors were co-expressed after 7 days *in vitro* by somites.

Conclusion: We concluded that neural induction property of somites appeared by production of required neural differentiation factors including cerberus, chordin, FGF8, follistatin and noggin.

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Keywords: Cerberus protein, Chordin, FGF8 protein, Follistatin, Noggin protein, Somites

* **Corresponding authors:** Kamran Ghaedi, Ph.D., and Mohammad Hossein Nasr-Esfahani, Ph.D., Department of Cellular Biotechnology at Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran
Tel: +98 311 9515694
Fax: +98 311 9515687
E-mail: kamranghaedi@royaninstitute.org; mh_nasr@royaninstitute.org
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Introduction

Somites are transient developmental structures and the derivatives of paraxial mesoderm which have an important role in organization of segmented pattern of vertebrate embryos¹. *In vitro* simulation of developmental mechanisms of vertebrates, especially mammals, could help us to find out the intrinsic molecular events involved in the development of organs and differentiation of various kinds of cells like CNS neurons. It is almost impossible to isolate developmental structures such

as somite and notochord in human to study their functions *in vitro*. Even in laboratory mammals like mice, somites are too small to isolate and meticulous work should be done for separating them. Thus, the sole way is to separate such structures in chicken². In such situation, chick embryos are the best choice for somite isolation and *in vitro* simulation of developmental process of neurons. This simulation helps us to find out whether human embryonic cells respond to the molecular sig-

nals sent by somite, thereby exploring a new way for regeneration of damaged neural tissues. Previous studies³ have shown that co-culture of somites derived from chick embryos of stages 9-12 of Hamburger and Hamilton⁴ caused an increase in TUJ1/HOXB4 double positive group among human neural progenitors. Also it has been shown by Sagha *et al*⁵ that somites maintain their neural induction ability in mouse embryonic stem cells. They have shown that somites thicken the adjacent part of neural tube, thereby affect the proliferation of neural tube precursors⁶.

So far, there is no confirmation on expression of secretory factors by somite, whether they are able to co-express several neurogenic factors like noggin, chordin, follistatin, cerberus and FGF8 *in vitro*. The neurogenic activity of noggin⁷, chordin⁸, follistatin⁹, cerberus¹⁰ and FGF8¹¹ has already been established. Thus, this study investigated the co-expression of the mentioned factors by somites at mRNA level.

Materials and Methods

Preparation of somite-containing alginate beads

Commercial chick eggs were provided through commercial sources and incubated in a humidified atmosphere at 38°C to yield the embryos at stages 9-12 as already described¹². Chick embryos were isolated from the yolk

surface and transferred into Leibovitz's (L15) medium (Invitrogen, USA). Then, embryos were dipped in dispase solution containing 1 mg dispase per 1 ml PBS (Invitrogen, USA) for 3-5 min for loosening chick embryo tissues. The enzyme was removed and embryos were washed with L15 medium supplemented with 5% Fetal Calf Serum (FCS; Invitrogen, USA) for 15 min. Subsequently, embryos were transferred into cold FCS free L15 medium. Somites were isolated from embryos under a dissecting microscope and transferred to medium. The presence of alginate beads facilitates the diffusing of secretory products of somites into the medium^{13,14}. These alginate beads helped somites to retain their integrity *in vitro* without somite cell migration in our media as well as to continue their intrinsic gene expression and protein secretion activity. Alginate beads were prepared according to previous reports⁵.

RNA isolation and RT-PCR

Total RNA of the somites was extracted using an RNeasy kit (Qiagen, Germany) according to manufacturer's protocol. cDNA synthesis was done using a cDNA synthesis kit (TaKaRa, Japan) according to manufacturer's instructions. Primer information is shown in table 1. PCR products were analyzed by gel electrophoresis on 1.5% agarose gel and stained with ethidium bromide (10 µg/ml), visual-

Table 1. Primers list in this study

Gene	Primer sequence (5'→3')	AT (°C)	Length	Accession No.
Cerberus	F: TCCTGCCAATCAAGACCAATG R: GTTCTGGACTATCACCTTCTCAC	58	104 bp	NM_204823.1
Chordin	F:GCACAGAGGAGCAGGGATG R:TACAAGGCGGGCAGATG	64.4	161 bp	NM_204980.1
FGF8	F:CGAGACCGACACCTTTGG R:TCCTTGCCTTTGCCGTTAC	55	114 bp	NM_001012767.1
Follistatin	F:CTTATCCGAGCGAGTGTG R: GTAGTCCTGGTCTTCATCTTC	58	134 bp	NM_205200.1
Noggin	F:CCCTAACTTTATGGCTATGTCCCT R: CCGCAGCAGCAAGTCCAG	60	79 bp	NM_204123.1

F and R: are forward and reverse primers respectively. AT is annealing temperature which was set for the PCR for each primer pair. The length is related to the size of amplified product which is a partial segment of the coding sequences of the respected genes. Accession No refers to the registered No of each respected mRNA

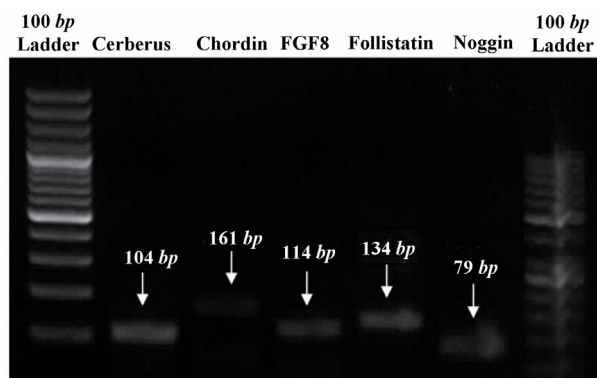


Figure 1. PCR products which were analyzed by gel electrophoresis evidencing that mentioned genes were expressed by chick somites *in vitro*

ized and photographed by a UV transilluminator (Uvidoc, UK) (Figure 1).

Results

RT-PCR on somite-derived cells revealed identifiable expression of cerberus, chordin, FGF8, follistatin and noggin after 7 days (Figure 1). The respective bands were not detected in no-template and no-primer tubes (data not shown).

Discussion

This finding was strongly representing that these factors may be translated and exert a specific role in neural differentiation. Therefore, it seems that somites could retain their *in vitro* ability for expression of such factors at mRNA level.

In another study, it was reported that noggin 4 was expressed during the early development of the chick embryo even in gastrulation¹⁵. This finding is consistent with our data that chick somite could express noggin. According to previous studies, it seems that neural induction ability of chick somite in human neural precursor cells³ could be due to the expression and production of one of these factors: FGF8, chordin, cerberus, follistatin or noggin. However, due to presence of trace amounts of numerous factors in somite, detection of these factors requires a meticulous proteomic approach or secretome analysis of somites to find the real candidates responsible

for induction of neural differentiation by somites.

In this study, we performed a conventional RT-PCR method as described to ensure that all aforementioned factors could be co expressed appropriately by chick somites.

Conflict of Interest

This study was approved by the ethical committee of Royan Institute. None of the authors has any conflicts of interest to disclose and all authors confirm the submission to this journal.

References

1. Gilbert SF. Developmental biology. 9th ed. Sunderland, Mass: Sinauer associates; 2010.
2. Rowan AM, Stern CD, Storey KG. Axial mesoderm refines rostrocaudal pattern in the chick nervous system. *Development* 1999;126(13):2921-2934.
3. Salehi H, Karbalaie K, Salamian A, Kiani A, Raza-vi S, Nasr-Esfahani MH, et al. Differentiation of human ES cell-derived neural progenitors to neuronal cells with regional specific identity by co-culturing of notochord and somite. *Stem Cell Res* 2012;8(1):120-133.
4. Hamburger V, Hamilton HL. A series of normal stages in the development of the chick embryo. *J Morphol* 1951;88(1):49-92.
5. Sagha M, Karbalaie K, Tanhaee S, Esfandiari E, Salehi H, Sadeghi-Aliabadi H, et al. Neural induction in mouse embryonic stem cells by co-culturing with chicken somites. *Stem Cells Dev* 2009;18(9):1351-1360.
6. Wilson L, Maden M. The mechanisms of dorso-ventral patterning in vertebrate neural tube. *Dev Biol* 2005;282(1):1-13.
7. Lim DA, Tramontin AD, Trevejo JM, Herrera DG, García-Verdugo JM, Alvarez-Buylla A. Noggin antagonizes BMP signaling to create a niche for adult neurogenesis. *Neuron* 2000;28(3):713-726.
8. Sasai Y, Lu B, Steinbeisser H, De Robertis EM. Regulation of neural induction by the Chd and Bmp-4 antagonistic patterning signals in *Xenopus*. *Nature* 1995;376(6538):333-336.
9. Urshansky N, Mausner-Fainberg K, Auriel E, Regev K, Karni A. Low and dysregulated production of follistatin in immune cells of relapsing-remitting

- multiple sclerosis patients. *J Neuroimmunol* 2011; 238(1-2):96-103.
10. Yu X, He F, Zhang T, Espinoza-Lewis RA, Lin L, Yang J, et al. Cerberus functions as a BMP agonist to synergistically induce nodal expression during left-right axis determination in the chick embryo. *Dev Dyn* 2008;237(12):3613-3623.
 11. Lahti L, Saarimäki-Vire J, Rita H, Partanen J. FGF signaling gradient maintains symmetrical proliferative divisions of midbrain neuronal progenitors. *Dev Biol* 2011;349(2):270-282.
 12. Salehi H, Karbalaie K, Razavi S, Tanhaee S, Nematollahi M, Sagha M, et al. Neuronal induction and regional identity by co-culture of adherent human embryonic stem cells with chicken notochords and somites. *Int J Dev Biol* 2011;55(3):321-326.
 13. Wells LA, Sheardown H. Extended release of high pI proteins from alginate microsphere via a novel encapsulation technique. *Eur J Pharm Biopharm* 2007;65(3):329-335.
 14. Anjomshoa M, Karbalaie K, Mardani M, Razavi S, Tanhaei S, Nasr-Esfahani MH, et al. Generation of motor neurons by coculture of retinoic acid-pre-treated embryonic stem cells with chicken notochords. *Stem Cells Dev* 2009;18(2):259-267.
 15. Borodulin AV, Eroshkin FM, Bayramov AV, Zarskiy AG. Noggin4 expression during chick embryonic development. *Int J Dev Bio* 2012;56(5): 403-406.