

# Induction of Alpha-Crystallins Expression in Umbilical Cord Mesenchymal Stem Cells

Masoud Maleki, PhD<sup>1</sup> • Kazem Parivar, PhD<sup>2</sup> • Mohammad Nabiyouni, PhD<sup>3</sup>  
Parichehr Yaghmaei, PhD<sup>2</sup> • Mohammad Naji<sup>3</sup>

---

## Abstract

**Purpose:** Our aim was to isolate mesenchymal stem cells (MSCs) from mouse umbilical cord and culture them with vitreous body to investigate their capacity to differentiate into lens fiber cells, crystallins are expressed.

**Methods:** Wharton's jelly is the appropriate source of MSCs. After isolation, the cells were cultured and alkaline phosphatase detection kit was used for staining undifferentiated umbilical cord-MSCs. In the experimental groups, MSCs were plated in the maintenance medium supplemented with bovine vitreous body (1:1, 3:1 v/v) for induction up to 10 days. Light microscopy and immunostaining methods used to assess differentiation of the cells.

**Results:** Mouse umbilical cord-MSCs have alkaline phosphatase activity and vitreous body can induce them to differentiate into the lens fiber cells.

**Conclusion:** Lens fiber cells can be produced by umbilical cord-MSCs derived from mouse. MSCs have the potential to be useful source for stem cell researchers.

**Keywords:** Crystallin, Lens Fiber Cells, Mesenchymal Stem Cells, Vitreous Body

*Iranian Journal of Ophthalmology 2010;22(2):67-71 © 2010 by the Iranian Society of Ophthalmology*

---

- 
1. Department of Biology, Ardabil Branch, Islamic Azad University
  2. Department of Biology, Tehran Science and Research Branch, Islamic Azad University
  3. Department of Biology, Tarbiat Moallem University

Received: December 29, 2009

Accepted: April 22, 2010

Correspondence to: Masoud Maleki, PhD

Department of Biology, Ardabil Branch, Islamic Azad University, Ardabil, Iran, Tel:+98 451 7729822, Email: maleki.masoud@gmail.com

## Introduction

Studies carried out over the past two decades have made possible a more exact definition of the stem cells, in which they are capable of self-renewal, can differentiate into multiple lineages, and will function *in vivo*.<sup>1</sup> A less examined source for mesenchymal stem cells (MSCs) is the stroma of umbilical cord, also called Wharton's jelly, originating from extraembryonic mesoderm at day 13 of human embryonic development.<sup>2</sup> Umbilical cord is composed of two arteries and one vein, all of which are surrounded by a unique connective tissue stroma rich in proteoglycans and mucopolysaccharides.<sup>3</sup> In recent studies, cord stromal cells were reported to encompass MSC character.<sup>4,5</sup> Wang et al<sup>4</sup> induced the differentiation of umbilical cord stromal cells into mesenchymal cell lineages, such as cardiomyogenic, chondrogenic, osteogenic, and adipogenic types. Sarugaser et al<sup>6</sup> showed the isolation, culturing, and differentiation behavior of human perivascular umbilical cells and obtained osteogenic nodules. Fu et al<sup>7</sup> and Weiss et al<sup>5</sup> demonstrated the differentiation capacity of human umbilical cord MSCs into dopaminergic neurons. Carlin et al<sup>8</sup> demonstrated some of embryonic stem cell markers, such as Oct-4, Sox-2, and Nanog, for the first time in porcine umbilical cord matrix cells. For Wharton's jelly cells to be considered an alternative and potential source of stem cells, a greater effort must be made to study these cells to determine their differentiation potency and whether they can serve as a prospective reserve of stem cells to use in cell-based therapies. There is no study about mouse umbilical cord stromal cells ability to differentiate into lens fiber cells. Lens is composed of two populations of cells: a layer of cuboidal epithelial cells covers the anterior surface, and elongated, terminally differentiated fiber cells comprise the bulk of the tissue. Epithelial cells proliferate and differentiate into fiber cells near the lens equator under the control of factors from outside the lens.<sup>9</sup> In differentiation pathway of lens fiber cells, crystallins are expressed. Crystallins, divided into two major families  $\alpha$  and  $\beta\gamma$  are the major proteins of the lens. Crystallins are known to constitute about 90% of water soluble proteins of the lens and contribute to the transparency and refractive

properties by a uniform concentration gradient in the lens. One of the most exciting findings in lens research has been the study of  $\alpha$ -crystallin as a molecular chaperone.<sup>10</sup>  $\alpha$ B-crystallin, but not  $\alpha$ A, is a stress-inducible protein, and both are members of the heat shock protein family.  $\alpha$ A and  $\alpha$ B;  $\beta$ B1,  $\beta$ B2,  $\beta$ B3,  $\beta$ A1/A3,  $\beta$ A2, and  $\beta$ A4;  $\gamma$ A,  $\gamma$ B,  $\gamma$ C,  $\gamma$ D,  $\gamma$ D,  $\gamma$ E,  $\gamma$ F and  $\gamma$ S are the major crystallins expressed in the mammalian lens.<sup>11</sup> The transcripts for crystallins appear very early during mouse lens development, with  $\alpha$ B-crystallin being detected in the lens placode at E9.5 in the mouse, and  $\alpha$ A-crystallin detected in the lens cup at E10 to 10.5.<sup>12</sup> Wharton's jelly is the gelatinous connective tissue from umbilical cord and is composed of myofibroblast-like stromal cells, collagen fibers, and proteoglycans.<sup>13</sup> Here, we present our findings about proliferative cells that we isolated from Wharton's jelly. Our results show that Wharton's jelly cells are easily attainable and can be expanded *in vitro*, maintained in culture, and induced to differentiate into lens fiber cells.

## Methods

### Isolation and cell culture

We isolated MSCs from the whole mouse umbilical cord. After having been minced into 1-2 mm<sup>3</sup> fragments, were washed with Hank's balanced salt solution (HBSS) then were incubated with collagenase type IA (Sigma, St Louis, MO, USA) for 10 min and then 2.5% trypsin (Sigma, St Louis, MO, USA) for 5 min following pipetting for mechanical isolation of the cells. Cells were plated at a density of  $1 \times 10^6$  cells/cm<sup>2</sup> in non-coated T-25 cell culture flask (Greiner bio-one, Germany). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich, St Louis, USA) supplemented with 10% Fetal bovine serum (FBS; Sigma-Aldrich, St Louis, USA) 1% penicillin/streptomycin (Sigma). Cultures were maintained in a humidified atmosphere with 5% CO<sub>2</sub> at 37°C. After 3 days of culture the medium was changed and nonadherent cells were removed. Once 60-80% confluence had been reached, adherent cells were subcultured.

### **Collection of vitreous body**

Bovine eyes were placed on ice immediately after harvesting and were transported to the laboratory. Vitreous fluid from 10 eyes was collected and pooled using a sterile syringe without a needle. The pooled vitreous was homogenized with a syringe and a 18-gauge needle following by centrifugation at 14000 rpm/4°C/20min, portioned into aliquots, and stored at -80°C.

### **Identification of stem cells**

Alkaline phosphatase activity is a marker for the phenotypic assessment of undifferentiated embryonic stem cells. Alkaline phosphatase detection kit (Chemicon; catalog number SCR004) was used for staining of undifferentiated umbilical-MSCs from passage 1(P<sub>1</sub>).

### **Induction of lens fiber cells**

In the experimental groups MSCs were plated in the maintenance medium, DMEM plus 10% FBS and 1% penicillin/streptomycin, supplemented with bovine vitreous body (1:1, 3:1 v/v) for induction, the supplemented medium was changed every two days.

### **Preparation of lysates**

After 10 days, culture flasks (experimentals and control) were placed on ice and the cells were washed with ice-cold phosphate buffer saline (PBS). Then ice-cold lysis buffer was added (20 mM Tris-HCl, 5 mM EDTA, pH 7.5) followed by scraping adherent cells using a cold plastic cell scraper, then gently cell suspensions were transferred into a pre-cooled microfuge tube and centrifuged at 12,000 rpm/4°C/20 min. Supernatants were aspirated and were stored at -80°C.

### **Western blot analysis of differentiated cells**

The expression of differentiation markers,  $\alpha$ A- and  $\alpha$ B- crystalline, were detected by Western blot analysis to standard procedure. Lysate of rat lens was used as a positive control. Cell lysates were subjected to electrophoresis (SDS-PAGE), and the separated proteins were transferred to nitrocellulose membrane (Fluka-biochemika: GA16285). Nonspecific antibody binding sites

were blocked by incubation with blocking buffer (5% BSA in 0.05% Tween 40, 50 mM Tris, 0.5 M NaCl) for 1 hour. The membranes were then incubated at room temperature with primary antibody diluted in blocking buffer for 1 hour. The rabbit polyclonal antibody, anti-alpha A + alpha B crystallin (1:1000; abcam) was used as primary antibody. The primary antibody binding was detected with a biotinylated anti-rabbit IgG made in goat (1:500; Vector Laboratories, Burlingame, CA). After washes with wash buffer (0.05% Tween 40, 50 mM Tris, 0.5 M NaCl) the membranes were incubated with vectastain ABC reagents (Standard Kit; Vector Laboratories, Burlingame, CA) and then were stained by vector blue alkaline phosphatase substrate kit III (Vector Laboratories, Burlingame, CA).

## **Results**

### **Morphological characteristics and alkaline phosphatase activity of MSCs**

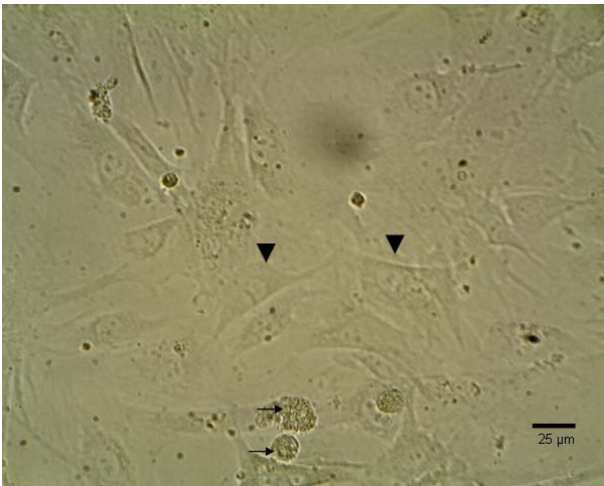
Freshly isolated cells (passage 0, P<sub>0</sub>) principally displayed a fibroblast-like appearance with long cytoplasmic processes extension as early as 24 hours (Figure 1). P<sub>1</sub> cells were examined for alkaline phosphatase marker expression; they showed alkaline phosphatase activity (Figure 2).

### **Morphological studies of experimental groups**

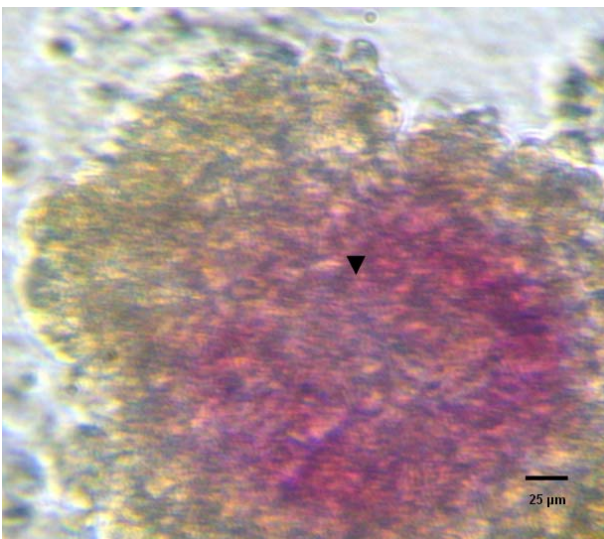
Light microscopy showed that most cells in experimental cells were locally long and parallel aligned and compared to control cells; their cytoplasmic processes decreased and had fiberlike appearance (Figure 3). The fiberlike cells had large nuclei with multiple nucleoli (Figure 3).

### **Analysis of Expression of $\alpha$ -crystallins in induced cells**

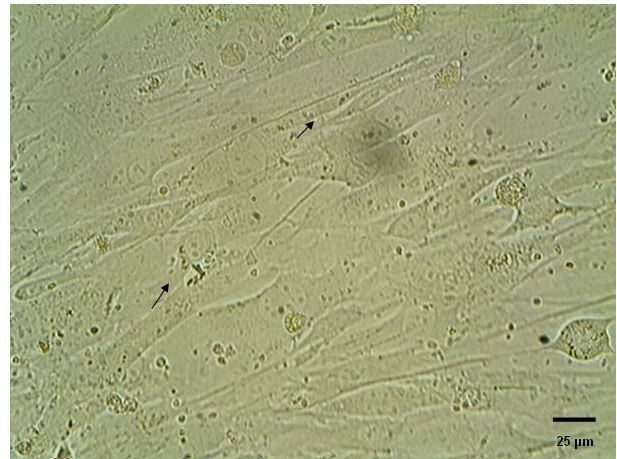
Western blot analysis of differentiated cells that were plated in the maintenance medium supplemented with bovine vitreous body (1:1, 3:1 v/v) showed that both  $\alpha$ A-crystallin and  $\alpha$ B-crystallin expressed in MSCs (Figure 4). Therefore, these cells proceeded in differentiation process to the similar stage of lens cup.



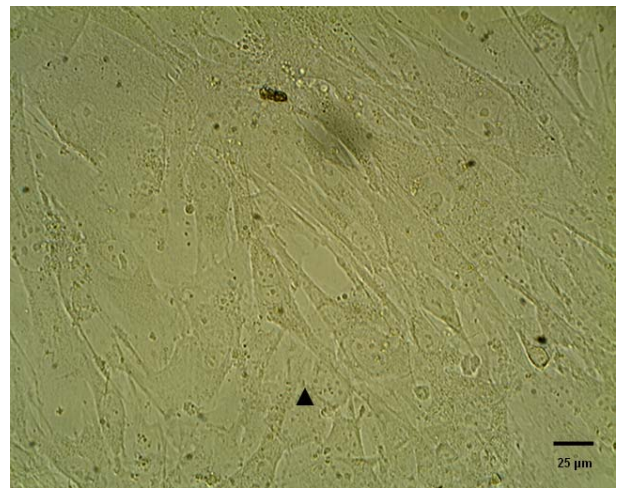
**Figure 1.** MSCs in the primary culture of whole mouse umbilical cord  
Fibroblastoid morphological aspect of MSCs is observed (arrowheads) and dead cells have been shown with arrows: 400X.



**Figure 2.** Alkaline phosphatase staining of umbilical cord MSCs  
Red region that are showed with arrowhead, revealed undifferentiated stem cells: 400X.

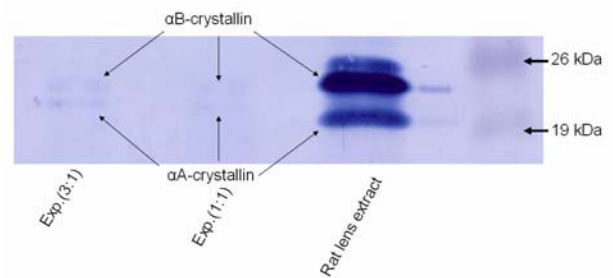


**A**



**B**

**Figure 3.** A. Experimental MSCs that cultured with maintenance medium supplemented with vitreous (1:1 v/v). Microscopical studies indicated that these cells are aligned, elongated and have fiberlike shape (arrow): 400X. B. Experimental MSCs that cultured with maintenance medium supplemented with vitreous (3:1 v/v). Microscopical studies indicate that locally these cells are aligned, elongated and have fiberlike shape (arrowhead): 400X.



**Figure 4.** αA- and αB- crystallin expression in experimental cultured MSCs by Western blotting. Rat lens extract served as positive control. Exp. (1:1) and Exp. (3:1): Experimental groups that have been plated in the maintenance medium supplemented with vitreous (1:1 v/v) and (3:1), respectively.

## Discussion

MSCs were initially isolated from bone marrow by Friedenstein et al<sup>14</sup> and then studied by other investigators.<sup>15,16</sup> Wharton's jelly is an alternative source to bone marrow for MSCs.<sup>17</sup> We report herein successful isolation of MSCs from whole mouse umbilical cord without its blood stem cells. Therefore umbilical cord derived cells that plated were from Wharton's jelly. The Wharton's jelly of the umbilical cord contains mucoid connective tissue and fibroblast-like cells. Using alkaline phosphatase detection kit, we found that predominant mesenchymal cells isolated from umbilical cord expressed alkaline phosphatase. This marker previously described for undifferentiated state of embryonic stem cells.<sup>18</sup> Because of alkaline phosphatase activity, we could speculate about pluripotency of umbilical cord derived MSCs. We investigated the potential of these cells to differentiate into lens fiber cells by treating them with vitreous. In the mouse,  $\alpha$ A-

and  $\alpha$ B- crystallin are detected in the lens cup.<sup>12</sup> Morphological studies showed that differentiated fiber-like cells were locally long and aligned. Western blot analysis of lens fiber cell markers,  $\alpha$ A- and  $\alpha$ B- crystallin, showed that they were expressed in both experimental groups but the level of expression in the group that MSCs were exposed to 25% vitreous as inducer were higher than those were exposed to 50% vitreous. Therefore, we can conclude that induced cells by vitreous, proceeded in differentiation up to similar stage of lens cup in the mouse embryo and the low level of inducer had better effect.

## Conclusion

Results presented in this study suggest that MSCs derived from mouse umbilical cord have the potential to be a useful source for stem cell researches.

## References

1. Verfaillie C. Adult stem cells: tissue specific or not? In: Lanza R, ed. *Essentials of Stem Cell Biology*, Oxford, UK: Elsevier Academic Press. 2006;23-7.
2. Sadler TW. Second week of development: Bilaminar germ disc, In: Sadler TW, ed. *Langman's Medical Embryology*. 9th ed. Philadelphia: Lippincott Williams & Wilkins, 2004;57 pp.
3. Sobolewski K, Bańkowski E, Chyczewski L, Jaworski S. Collagen and glycosaminoglycans of Wharton's jelly. *Biol Neonate* 1997;71(1):11-21.
4. Wang HS, Hung SC, Peng ST, et al. Mesenchymal stem cells in the Wharton's jelly of the human umbilical cord. *Stem Cells* 2004;22(7):1330-7.
5. Weiss ML, Medicetty S, Bledsoe AR, et al. Human umbilical cord matrix stem cells: preliminary characterization and effect of transplantation in a rodent model of Parkinson's disease. *Stem Cells* 2006;24(3):781-92.
6. Sarugaser R, Lickorish D, Baksh D, et al. Human umbilical cord perivascular (HUCPV) cells: a source of mesenchymal progenitors. *Stem Cells* 2005;23(2):220-9.
7. Fu YS, Cheng YC, Lin MY, et al. Conversion of human umbilical cord mesenchymal stem cells in Wharton's jelly to dopaminergic neurons in vitro: potential therapeutic application for Parkinsonism. *Stem Cells* 2006;24(1):115-24.
8. Carlin R, Davis D, Weiss M, et al. Expression of early transcription factors Oct-4, Sox-2 and Nanog by porcine umbilical cord (PUC) matrix cells. *Reprod Biol Endocrinol* 2006;4:8.
9. Coulombre JL, Coulombre AJ. Lens development: fiber elongation and lens orientation. *Science* 1963;142:1489-90.
10. Horwitz J. Alpha-crystallin can function as a molecular chaperone. *Proc Natl Acad Sci USA* 1992;89(1):10449-53.
11. Bloemendal H, de Jong W, Jaenicke R, et al. Ageing and vision: structure, stability and function of lens crystallins. *Prog Biophys Mol Biol* 2004;86(3):407-85.
12. Robinson ML, Overbeek PA. Differential expression of alpha A- and alpha B-crystallin during murine ocular development. *Invest Ophthalmol Vis. Sci* 1996;37(11):2276-84.

13. Kobayashi K, Kubota T, Aso T. Study on myofibroblast differentiation in the stromal cells of Wharton's jelly: expression and localization of alpha-smooth muscle actin. *Early Hum Dev* 1998;51(3):223-33.
14. Friedenstein AJ, Gorskaja JF, Kulagina NN. Fibroblast precursors in normal and irradiated mouse hematopoietic organs. *Exp Hematol* 1976;4(5):267-74.
15. Mets T, Verdonk G. In vitro aging of human bone marrow derived stromal cells. *Mech Ageing Dev* 1981;16(1):81-9.
16. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999;284(5411):143-7.
17. Lu LL, Liu YJ, Yang SG, et al. Isolation and characterization of human umbilical cord mesenchymal stem cells with hematopoiesis-supportive function and other potentials. *Hematologica* 2006;91(8):1017-26.
18. Pease S, Braghetta P, Gearing D, et al. Isolation of embryonic stem (ES) cells in media, supplemented with recombinant leukemia inhibitory factor (LIF). *Dev Biol* 1990;141(2):344-52.