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Original Article

Differentiation of Stem Cells derived from NMRI Mature Mouse Omentum to Kidney Cells by using Extract of Immature Mouse Kidney

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Abstract

During recent years, many successes were obtained in the field of stem cells. At future, the cells will be progressed into medical sciences and of most important successes are treatment cells methods. Today, treatment cells are regarded as focal point on medical fields. Stem cells are used to treat vast majority of diseases. In this study, Omentum is used as one of the production units of stem cells and is used to discriminate kidney cells from mouse extraction which contains growth factor.

Methodology: Omentum texture of NMRI mouse is obtained and was transferred into PBS dish and cultured into cellular flask. When first texture reached to 95% consent mouseion, different percent of kidney extraction transferred into cellular environment, proof of omentum stem cells was discussed by RT-PCR method.

Results: Discussion results of RT-PCT methods were recognized by stem cells and realized omentum cells. As well, by discussion RT-PCR, differentiation of stem cells was confirmed under extraction of kidney. The results are due to kidney extraction under omentum mesenchyme and effective dose of extraction was 10y which obtained significant cells with morphology kidney cells.

Key words: Stem Cells, Omentum, Extract of Immature Mouse Kidney, Differentiation of Cells

INTRODUCTION

Stem cell is origin and derivation of cells in body and has two specifications like Pluripotency and self- renewal that means they convert into kinds of cells and can convert into similar under discriminate cells to survive as main resource of stem cells(Watt et al.,2000). Depend on kind of stem cells, the cells have power to convert into one or more different cells and help to treat all different diseases (Tarfiei et al.,2009).

Kinds of stem cells are discriminated in terms of tolerance into reproduction and cellular differentiation and evolution step (Desseso et al., 2004)

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Mesenchyme stem cells (MSCs) were stated for the first time at 1966 in Fridenstein and Petrakova who successes to sepamouse ancestor cells for brain of stone (Barry et al.,2004).

And some of authors like Piersma and Owen et al. in 1980 found it (Piersma et al.,1985,Owen.,1988). The cells are derived from stem cells and found in the textures like brain of stone, skeleton muscle, fat texture, and umbilical cord blood and skin texture environment (Schwartz et al.,2003).

MATERIALS AND METHODS

In this experimental work, stem cells are sepamoused from omentum of NMT in weight of 25-30. In order to sepamouse omentum texture for spinal cord injury, Texture omentum is clear and transparent texture thus, blood texture and fat around it was sepamoused from main omentum and plate containing PBS was transferred. Then, in cold conditions, main sterile texture was removed and washed with PBS. Texture parts were obtained and in order to culture of stem cells, as well as filtering

cultures, there are many indicators which are maintained in incubator. Normally after culture of stem cells, cell texture environment was removed during 4 days and washed by PBS solution twice and DMEM solution was entered into cell texture environment. When stem cells were reproduced, each four days, extraction of NMRI mouse added and the cells were discussed by flosyometr method.

The results of morphologic cells indicated that after 19 hours from culture of cell and deletion of no adhesive cells were discussed and focal morphology of cells (figure 1) and concentmouseion and number of cells were slight (generally, sepamoused from each other) and then culture environment was discussed by PBS in cold flask and DMEM environment was investigated in order to FBS 01%. 48 hours after texture, many cells were appeared in flask (2) and gradually the cells are grown. As if in day 5th, colons are many (3) and after one week, reached into confluence and formed one single cellular flask and indicated mesenchyme cells (4), in order to maximization of cells, the cells are passaged and this increases speed of cells. During 10-15 days, next passage was done and the cells are freeze in third passage as necessary (5).

Also, the most important point is that after melting, again, the cells returned into dish and reduced their growth but mutual morphology and discriminate potential was maintained. Thus, maintenance of cells was possible in long term freeze situation and will be used to transfer gene, simulations and so on.

After culture of omentum cell, in order to discuss markers of stem cells and their confirmation by Flosymetry, the third passage was used. The results due to marker analysis for positive level of stem cell indicated that CD90, CD44 markers were expressed as 92.57% and 100% respectively. Stem cells are not expressed bloody typical cells means CD45 and the results showed that special parkers of stem cells are sepamoused from cells and confirmed quality of sepamouseion of MSCs.

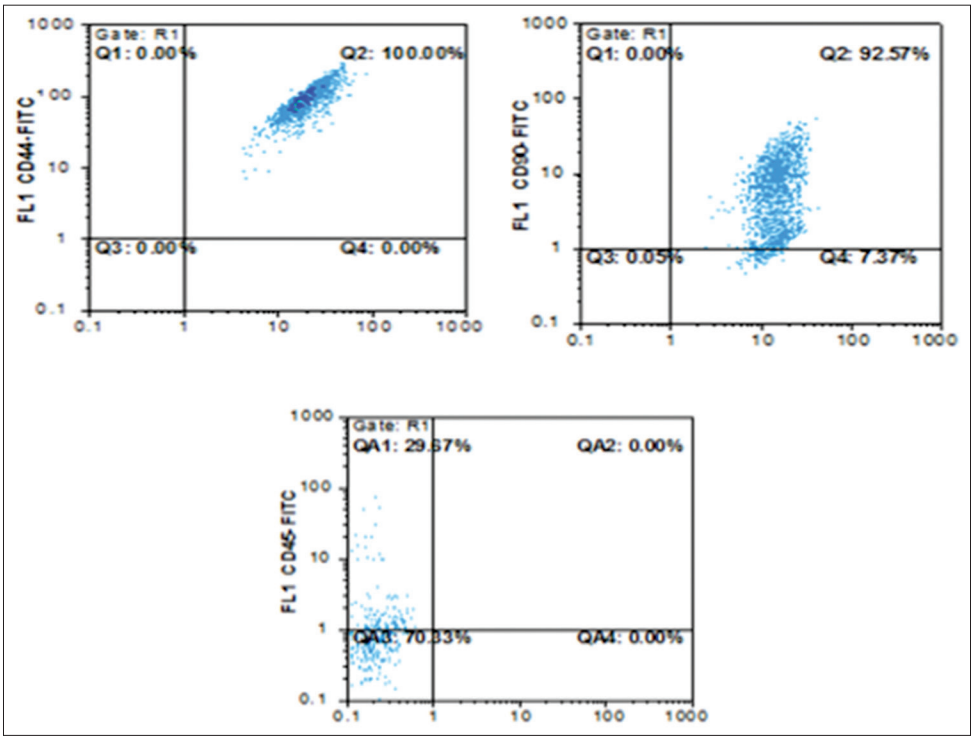
Flosymetry Analysis for Textured Cells

(A) Antibody CD90 92.57% having expression (B) and antibody CD44 in 100% of cells had expressed and (C) antibody Cd45 lacks expression

Results derived from effect of kidney extraction for differentiation of stem cells

Mesenchyme stem cells were under influenced by different doses of extraction. In this study, kidney extraction in doses 10, 3 and 50 were evaluated and mesenchyme cells were treated by kidney extraction in 4 days.

Treated mesenchyme cells were under 3-4 weeks by dose 10. In this dose, the highest scale for treatment is obtained in day 14th, direction and migmouseion of cells are performed (figure 1) and in day 21nd, cells showed progressive changes. In this day, adhesive cells are formed by ECM (figure 2) and did not discriminate by gene extraction and had slight effects (figure 3) and in day 34, the cells were analysed by morphological point of view (figure 5). In order to



discriminate omentum stem cells into kidney cells, RT-PCR was performed. In this method, kidney genes were proved.

Treated mesenchyme cells by dose 30 showed slight changes and were considered as control group and lacking cellular differentiation was observed by RT-PCR method.

RNA samples were extracted by kidney extraction from treated cells by dose 10. After discussion qualitative and quantitative methods by RNA and reaction of PCR BY USING starter markers were done by Pax2, Six 2, Wt1 and length of production part on gene 199 Pax 2 and 177 Six 2 and Wt1 were 212 pairs.

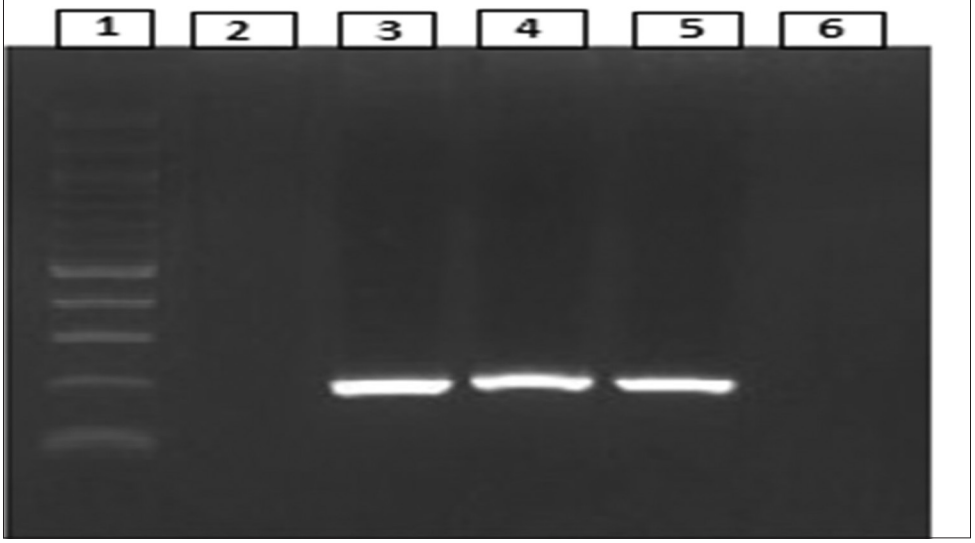
Consequent of Kindey Genes Primers

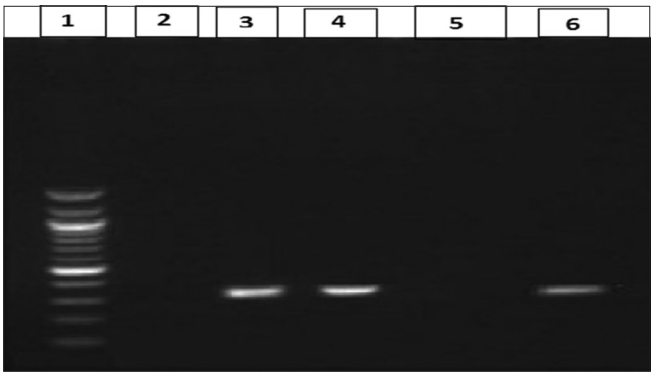
Figure 8, expression of gene Pax2, DNA ladder 2- untreated cell (negative control) sample, positive control, positive control, PCR negative control (H_2O)

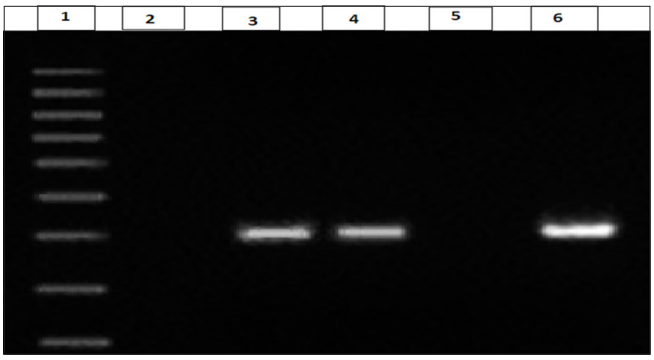
FIGURE 9, Six 2 gene expression, DNA ladder, omentum under treated cells (negative control), sample, positive control, PCR (H2O) negative control, positive control

Figure 10, Wt expression gene, DNA ladder, undertreated cells by Omentum (negative control), sample, positive

Gene Name	Gene Bank Number	Primer Sequence (5′ – 3′)	Product Size (bp)	Annealing Temperature (°C)
Pax2	NM_011037	Forward CTGCCCACATTAGAGGAGGT Reverse TGGAAGACATCGGGATAGGA	199	55
Six2	NM_009189	Forward AACCCCTACCCCTCACCG Reverse AGAGAGTTGATTCTGCTTGTT	177	55
Wt1	NM_144783	Forward GAGAGCCAGCCTACCATCC Reverse CAACTGTCAGTAGGGGTGTGG	212	61







control, PCR Negative Control (H_2O), Positive Control

DISCUSSION

Mature stem cells are under discriminated cells which are on texture; the cells are reproduced, In other words, self-renewable. Also, they can be special cells and play role of mature cells in life texture. One of the most important points in mature stem cells is the limited number of cells; the cells are on the normal mode and are in the classified mode. The cells are not classified as silent and calm mode and as necessary, they need restomouseion and destruction for a texture and play vital role by biotechnological modes. Because of limitation of cells, they can classify in each texture and environment and then produce kind of bond (Norbakhsh et al.,2010). One of the most important aims of stem cells is to find accessible resource and the results showed that omentum is available source for stem cells (Libermann.,2000)

Sepamouseion and summary process of stem cells are derived because of homogeny cells and this is derived from MSC to treat new treatment methods and texture engineering for all diseases. Extension and use of clinical functions was obtained by MSC method and it is due to physiologic and pathologic modes for all mammals (Dehghani Fard et al.,2011) which were used as model in the research.

Although, stem cells have high potential for three ectoderm, endoderm and mesoderm layers but it constrains application of stem cells to restore damages and treatment of diseases, whereas, mature stem cells have vast ability to discriminate in vivo and in vitro cells and the results proved to differentiation cells.

Most of the important specifications of stem cells is to ability adhesion into plastic surface. Of specifications are used to discriminate cells like bloody cells which lack specification. Morphological discussion of cells is multi shapes forms and other specifications are regarded as mesenchyme cells. In this study, it was recognized that cellular population are sepamoused from omentum texture and there is heterogeneous population containing 10% serum and other cells in the manner of cellular reproduction environment and also, first specification of formulation influences on cells differentiation. Original omentum contains biological materials like transferor and has growth factors which increase textures.

Omentum mesenchyme cells play vital role in treatment which was recognized by Cannday in 1948. In experiment by Sing and et al, it was recognized that stem cells migmoused into damaged environment (Solati.,2012).

The main specifications of mesenchyme cells, is to offer regulation specifications, protein synthesis and growth factor and secretion of safety materials. Sine omentum is full of neutrophil against pollution and prevents from infection; it can be applied in cellular texture and differentiation step.

In this study, discussion of stem cells was proved by RT-PCR method and in the test step, it was recognized that omentum has surface antigen like CD90+, CD44+ which reported as 93.57% and 100% but CD45- was not expressed. As result, it can be said that omentum cells are mesenchyme. Also results of RT-PCR showed that mature stem cells like Wt1, Oct 4, B-actin had expressed as 20bp, 169bp, 426bp which indicated and lost omentum cells as if it is regarded as threat for cells. Losing performance of kidney is regarded as a main indicator for exiting poisonous materials and volume and combination of fluids is defined as natural mode of body. At present, there are two main methods for combining insufficient of kidney and the applicants can limit them. Another dialyse method has many limitations. Because dialyse can be limited against it. Because dialyse cannot be replaced with it and was used to treat and use of stem cell can be helpful results and nearly 10-15% of American adult population are exposed to chronic kidney pathogen and it has kidney insufficiency because of multi facets which are derived from animals, this prevalence is 11.2% in Japan and 18.7% in Singapore and was reported as 10.1% in east Asia. In Iran, according to studies in 2009 by Hossein Panah et al in population 10063 ages 20 years old, prevalence of expose to kidney insufficiency was helpful and it seems that there is potential to stem cells for brain and foetus stem cells for treat kidney and texture engineering by Lazeri in 2007 and Guiilot 2008, Morigi and Herrera 2004. Stem cells were found in different organs and there are many reasons to determine differentiation of cells after damage (Fuchs et al.,2000). Maeshima and et al 2002 proved that reproductive cells are regarded as markers for pax2 and it is necessary evolution in terms of fetus which suggests immature phenotype.

Stem cells are regarded for main resource in treatment (Duffield.,2005, lin.,2005). Thus, there is main target for design new treatment methods and indicate stem cells (Chen.,2008, Plotkin.,2008, Goligorsky.,2006) developed first methods to sepamouse cells and study their specifications.

Da Silva Meirells and et al 2006 reported that stem cells are on many mature organs and stem cells of each texture is discriminated to other organs but the results by Qeysari and Soleymani indicated that stem cells are able to discriminate cells and also, Rezaneghad and Soulati studies demonst moused that omentum differentiation cells proved in

kidney and neural organ and our results indicated omentum stem cells have ability to regulate kidney cells.

In this research, cellular differentiation of MSC was proved by mouse cells and present research hypothesis indicated that there is suitable bed to different growth factors and it is possible that stem cells were facilitated by omentum cells and also, it was indicated that kidney cells were influenced under evolution environment and this growth factor can be important role in kidney morphological point of view.

The results obtained on kidney extraction in absence of growth factors and it was effective in omentum cells in differentiation mode by dose 10 which obtained by 3-4 weeks after cellular differentiation.

In this research, we could provide more fields to cellular differentiation by kidney cells and make differentiation effective factors in relation with factors.

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