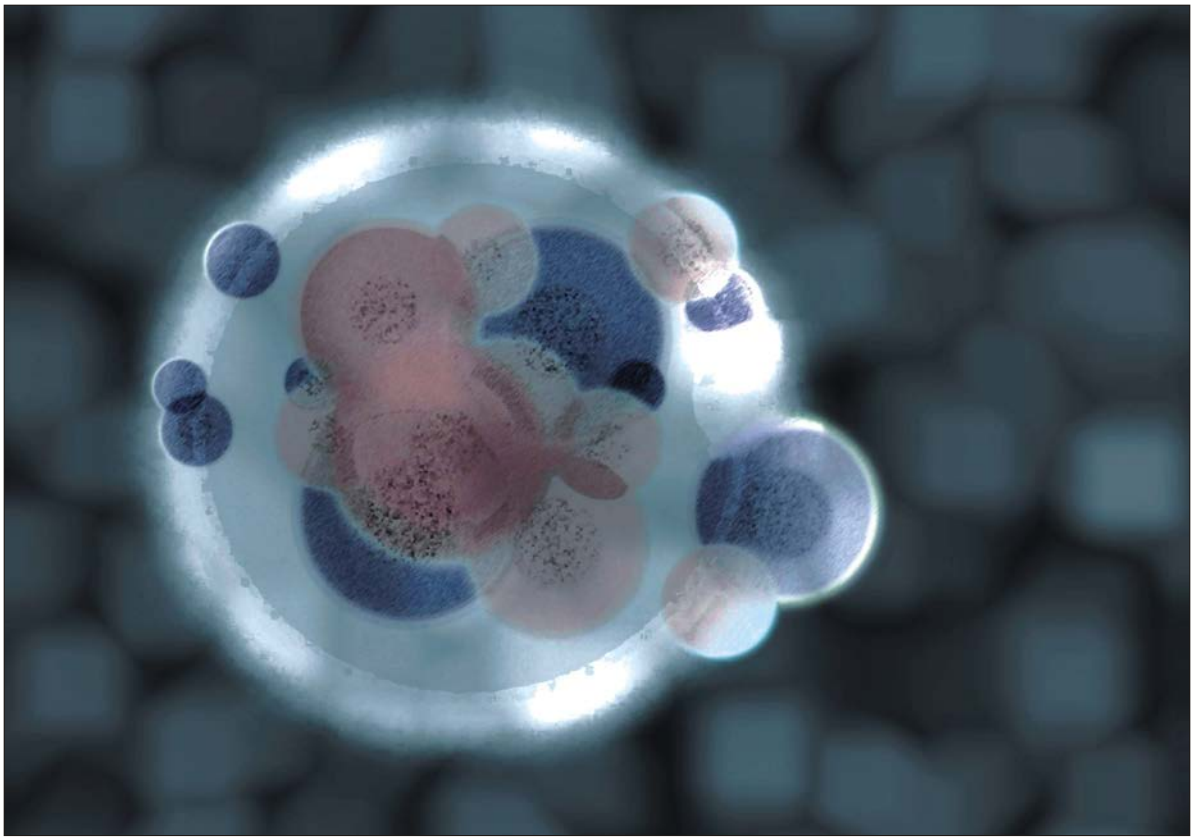

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Peritoneal Mesothelial Progenitor or Stem Cell

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Abstract: The aim of this study was evaluation of some specific markers of hematopoietic and mesenchymal stem cell and in mesothelial cells of peritoneum. In order to investigate the plasticity of Mesothelial Cells (MC), cell population from peritoneal dialysis fluid of early stage non-peritonitis male patients with 35-40 years old were first screened. Then they were analyzed by cell culture, flow cytometry and immunophenotyping for dominant markers such as CD45 and CD34 (as hematopoietic stem cell marker), HBME-1 (as mesothelial cell marker) and cytokeratin 18 (as an epithelial marker). Our results showed MCs possess some identical markers to mesothelial and epithelial cells. Also both mesenchymal ($CD34^-$) and hematopoietic ($CD45^+$) stem cell markers were detected in these cells. This study could change the previously assumed plasticity of mesothelial cells as progenitor cells and lead us to the definition of a new expression of surface markers of MCS.

Key words: Mesothelial, stem cell, CD34, CD45, cytokeratin 18, HBME-1

INTRODUCTION

Stem cells have unique capabilities to self-renew and differentiate or develop into multiple types of cells and tissues. While progenitor cells will give rise to a more restricted progeny of a stem cell (Wagers and Weissman, 2004).

The lateral mesoderm divides into somatic (parietal) and visceral (splanchnic) layers, which consequently give rise to the monolayer of mesothelial between the body wall (somatopleure) and the gut wall (splanchnopleure) (Herrick and Mutsaers, 2004). The monolayer of the mesothelium is supported by connective tissue consisting of fibroblast, collagen fibers, adipocyte, leukocytes and an abundant supply of lymphocytes and micro vessels (Witz *et al*., 1998). The presence of tight junctions and desmosomes has been reported (Di Poala and Sacchi, 1990).

The MCs from normal serosal tissue or fluid show cobblestone epithelial morphology in culture, however they can change through passages or continuous peritoneal dialysis to fibroblastic phenotype and undergo epithelial mesenchymal transition, which is characterized by a reduction in cytokeratin and E-Cadherin and on the other hand with an increase in integrin and vimentin expression (Herrick and Mutsaers, 2004; Witz *et al*., 1998; Yanez-Mo *et al*., 2003; Comin *et al*., 2006; Segers *et al*., 2006). Masothelial cells express cytokeratin 18, 19, 8, vimentin and desmin (Herrick and Mutsaers, 2004), HBME-1 (Volante *et al*., 2004; Sun *et al*., 2001; Frierson *et al*., 2003), CD31 (Darai *et al*., 1998; Sapino *et al*., 2001) CD34 (Lee *et al*., 2006), but it is not clear in what state of cell transition *in vivo* or *in vitro* they express these markers (Herrick and Mutsaers, 2004; Yanez-Mo *et al*., 2003; Venable *et al*., 2005). Evidence supporting the existence of peritoneal mesothelial cell as stem cell is still immature. Recently, based on observations made in human as well as in laboratory animals showing bone, bone marrow, cartilaginous tissue, glomerular-like structures and creation of artificial blood conducts resulting from pathological situations (mesothelioma, sclerosing peritonitis) suggested the presence of pluripotent mesenchymal cells in the mesothelial monolayer (Gotloib *et al*., 2007). They believed pluripotent mesenchymal cells in the mesothelial monolayer as well as in the submesothelial connective tissue raise the possibility of using the peritoneal mesothelium in regenerative therapies.

Peritoneum derived progenitor/stem cells isolated from peritoneal dialysis fluid bear a strong investigates as demonstrated by their expression of common surface markers to make them as stem cell (Gotloib *et al*., 2007).

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We first screened cell population in the peritoneal dialysis fluid from non peritonitis patients. Then after several passages CD45, CD34, HBME-1 and cytokeratin 18 markers were analyzed by cell culture, flow cytometry and immunophenotyping.

MATERIALS AND METHODS

Cell culture: Peritoneal dialysis fluid was attained from early stage male patients (old; 30-40) undergoing peritoneal dialysis from Tale ghani Hospital of Shahid Beheshti Medical Science University. Patients showed no clinical symptoms of peritonitis and were between 30-40 years old. Throughout peritoneal dialysis a catheter is inserted between visceral and parietal membranes of the peritoneum. Cells were centrifuged at 1500 rpm for 5 min and then cultured in DMEM, 15% FCS, 100 u mL^{-1} of penicillin and 100 μU mL^{-1} of Streptomycin.

Flow cytometry: Having discarded the non adherent blood cells after one subculture, adherent cells were trypsinized and centrifuged. Cells were washed in PBS and blocked with 5% goat serum for 30 min at room temperature. Excess blocker was washed with PBS. Mouse anti human HBME-1 (ab2383; Abcam, UK) monoclonal primary antibody, CD34 (ab6330; Abcam, UK) and CD45 were used in separate experiments diluted to 200 and incubated for 1 h at room temperature. The FITC conjugated goat anti mouse polyclonal secondary antibody (1:1000) (Ab6785; Abcam,UK) was incubated for 30 min at room temperature. Cells were analyzed by fluorescent microscopy (Axioskop 2 plus, Zeis) and flow cytometry (Becton Dickinson, FACS Calibur).

Immunophenotyping: Cells at sub-confluence on chamber glass slides were fixed in acetone for 5-10 min at room temperature, washed with phosphate-buffered saline (pH 7.4) and processed for immunocytochemical staining. Immunocytochemical staining was performed according to the manufacturer's instructions for each monoclonal antibody.

Cytokeratin 18 expression was determined by immunophenotyping method.

RESULTS

Peritoneal dialysis fluid samples were obtained from early stage male patients (between 35-40 years old, n = 15) from Taleghani Hospital of Medical Sciences University of Shahid Beheshti. Patients showed no clinical symptoms of peritonitis. They were examined immunofluorescence method for the expression of mesothelial marker HBME-1 (9), hematopoietic marker CD34 and CD45. Representative mesothelial cell cultures grown from peritoneal effluents of early stage on PD in 1st passage has shown in Fig. 1a. Phase-contrast microscopy showing different morphology of MCs in culture. The photomicrographs show the intermediate phenotype (some cobblestone like and some fibroblast like phenotype). The 4th passage showed fibroblast cell like appearance (Fig. 1b). HBME-1, a dominant marker of MCs, was detected in isolated cells of peritoneal dialysis fluid (Fig. 2). The majority of cells appeared this marker.

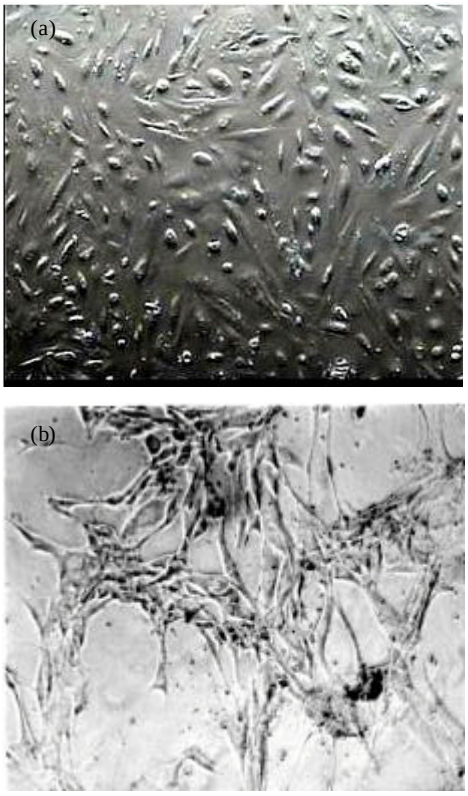

Fig. 1: *In vivo* MC cultures, grown from peritoneal effluents of early stage on PD in 1st passage. Phase-contrast microscopy showing different morphology of MCs in culture. The photomicrograph (a) shows intermediate phenotype (not absolute cobblestone like and not absolute fibroblast like phenotype (magnification: x100)). The photomicrograph (b) shows mesenchymal like appearance in 4th passage (magnification: x200)

CD34 were positively identified in the 22% of cells (passage 1), 2.45% (passage 4) and CD45 expression was observed in 16.28% of cells with immunofluorescence staining.

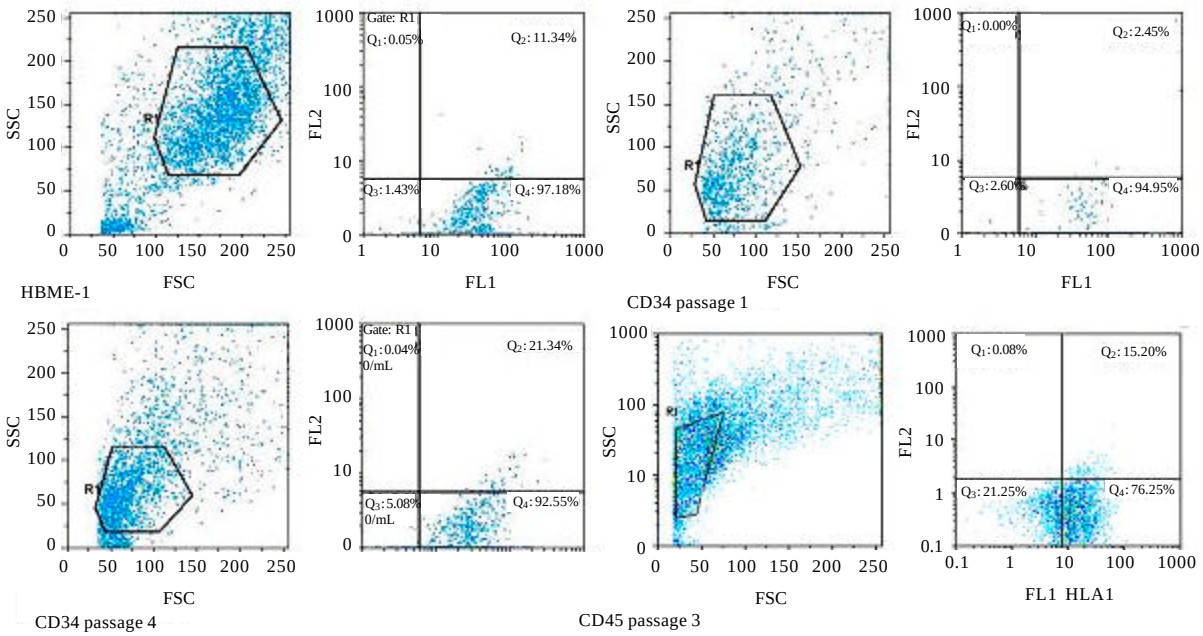

Fig. 2: Screening for cell populations of the peritoneum fluid: Flow cytometry of cell populations show a HBME-1, CD34 (at passage 1), CD45, positive population and CD34 at passage 3-4 represent minor populations. Control group was attained from fibroblasts skin from the same patients

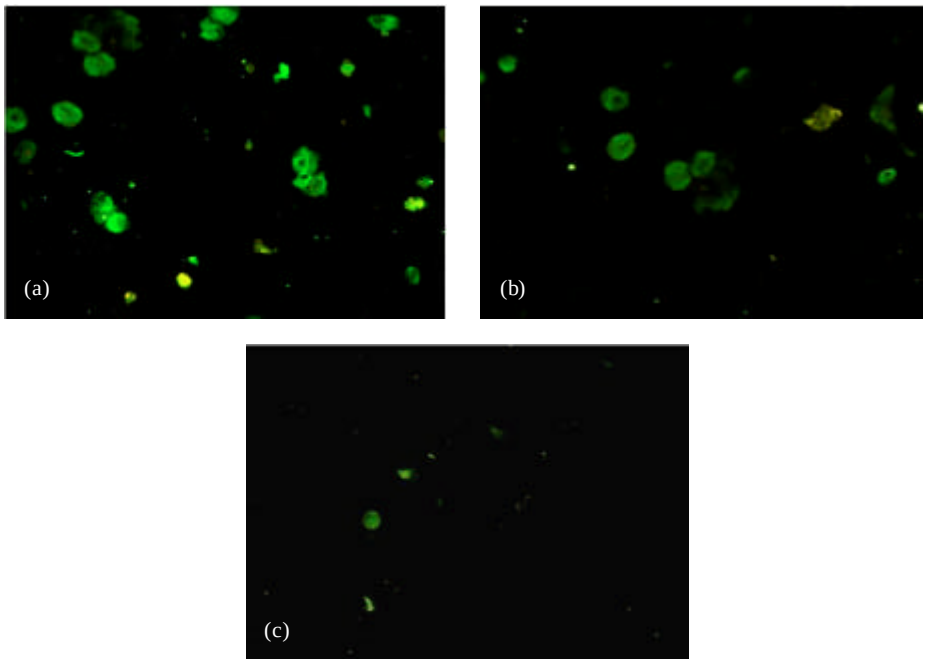

Fig. 3: Immuno-staining performed on the PD cultured cells before (a) 1st passage, (b) 4th passage and (c) control group

Immunophenotyping staining of CK18 performed on the peritoneal dialysis cultured cells immediately (a) before culturing MCs, (b) after first passage and (c) 4th passage has shown in Fig. 3a-c. Present data indicates expression of cytokeratin 18 to decrease gradually during passages 3-4.

DISCUSSION

In the present study, CD34 marker which is specifically expressed by hematopoietic stem cells was emphasized in peritoneal mesothelial cells. On the other hand, it has been shown endothelial cells lining the blood vessels are CD34 positive cells too. In addition to endothelial cells lining the blood vessels; there are CD34 positive cells in the stroma of human processus vaginalis peritonei (Roselini *et al*., 2007). This perhaps is explained by migration of these cells into the peritoneal cavity. Therefore the mutual expression of CD34 by MCs lineage confirms this theory. Present data showed the expression of CD34 gradually disappears in passages 3-4. The significance of the loss of CD34 expression is unknown. Based on some experimental results it seems to correlate with the appearance of the myofibroblastic phenotype (Jimenez-Heffernan *et al*., 2004). Tissue $CD34^+$ fibroblasts are closely related to circulating $CD34^+$ fibrocytes and reflect a bone marrow origin (Aroeira *et al*., 2007).

CD34 and CD45 positive cells has long been regarded as a reliable marker for Hematopoiectic Stem Cells (HSC), but recent studies have demonstrated the existence of CD34 negative HSC and that the two populations of HSC ($CD34^+$ and $CD34^-$) can differentiate into the other (Guiting *et al*., 2008). This loss of CD34 expression is probably due to down regulation of CD34 expression rather than death of $CD34^+$ cells. It seems they are simply residual embryonic mesenchymal cells that remained in the peritoneal tissue after organogenesis.

In the present study, cell populations within the peritoneal fluid displayed immunoreactivity for HBME-1, MCs marker (Roselini *et al*., 2007) and cytokeratin 18, an epithelial marker (Mutsaers, 2004), in primary culture. Indeed they showed double epithelial/mesenchymal phenotype. Our results were agreed to findings of Rosellini *et al*. (2007). After the third passage, the cells lost CK18 expression completely and the epithelial phenotype was only confined to a small fraction of cells. It can be reflected the switch of differentiation from a double epithelioid-mesenchymal phenotype towards predominantly mesenchymal fibroblast-like cells.

In support of this hypothesis, recent studies have been shown MCs undergo an epithelial to mesenchymal transition and transformation into myofibroblasts and possibly smooth muscle cells (Roselini *et al*., 2007; Guiting *et al*., 2008; Gronthos *et al*., 2003; Yung *et al*., 2006).

What is apparent is that population of pluripotent stem cells reside in the peritoneum from mesothelial origin that retain their undifferentiated state *in vivo* probably in an undefined microenvironment, but demonstrate plasticity in regenerating the peritoneum in state of injury and trauma, as previous studies reveal a considerable proliferation capacity among MCs (Herrick and Mutsaers, 2004).

What is obvious from this study is that MCs retain a multipotent or pluripotent capacity of differentiation to further than mesothelial derived cell lines.

As obtaining MCs from peritoneum is more clinically feasible and less invasive than bone marrow aspiration this source of stem cells could be an alternative source rather than bone marrow stem cells. The further capacity of MCs in differentiation to various cell lines should also be investigated. Due to the ethical concerns limiting manipulation of embryonic stem cells, adult stem cells have shown applicability in use in tissue engraftments, cell and gene therapy, repair and tissue regeneration.

Thus, the definition of a novella resource of adult stem cells definitely renders a massive impact on regenerative biomedicine. Future research will address the electrophysiological and clinical study of differentiated cells from this source. Furthermore, the full differentiation potential of mesothelial stem cells will also be chanllenged in further *in vitro* differentiation studies.

It seems peritoneal MCs in culture possess immunocytochemical markers identical to mesothelial stem cells, therefore culture of MCs offers researchers an essential tool to assess their morphologic, structural and functional structure. This study could violate the previously assumed plasticity of mesothelial progenitor cells and probability lead us to the definition of a new source of stem cells.

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