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Article *in* Cell Biology International · April 2014

DOI: 10.1002/cbin.10231 · Source: PubMed

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Cell Biology International ISSN 1065-6995
doi: 10.1002/cbin.10231

SHORT COMMUNICATION

In vitro differentiation of mesenchymal stem cells into mesangial cells when co-cultured with injured mesangial cells

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Abstract

Mesangial cells are one of the three major cell types of the kidney glomerulus that provide physical support for the glomerular capillary lumen of the kidney. Loss of mesangial cells due to pathologic conditions, such as glomerulonephritis and diabetic nephropathy, can impair renal function. Mesenchymal stem cells (MSC) are attractive candidates for kidney repair therapy since they can enhance recovery and protect against kidney failure. MSC can differentiate into mesangial cells in vivo. We have investigated the ability of MSC to differentiate into mesangial cells in vitro; they were co-cultured with oxidant-injured mesangial cells before being analysed by flow cytometry and for contractility. MSC co-cultured with injured mesangial cells had a mesangial cell-like morphology and contracted in response to angiotensin II. They expressed $CD54^{-}$ $CD62E^{+}$ in direct contrast to the $CD54^{+}$ $CD62E^{-}$ of pure MSC. In conclusion, MSC can differentiate into mesangial cells in vitro when co-cultured with injured mesangial cells.

Keywords: co-culture; in vitro differentiation; mesenchymal stem cell; mesangial cell; kidney injury

Introduction

The plasticity of bone marrow-derived mesenchymal stem cells (MSC) gives them the potential for multidirectional differentiation that is not limited to cells homologous within mesoderm lineage cell types, such as adipocytes, chondrocytes and osteocytes. MSC can differentiate into other lineages, such as neurons (ectoderm lineage cell type) and liver cells (endoderm lineage cell type) (Schwartz et al., 2002; Naghdi et al., 2009).

Kidney failure is a common and largely treatment-resistant clinical problem with an unacceptably high mortality rate, mainly due to the ineffectiveness of current therapies. Mesangial cells are one of the three major cell types of the kidney glomerulus, whose primary function is to provide physical support for the glomerular capillary lumen of the kidney (Mene and Stoppacciaro, 2009). These cells are located in the intraglomerular structure and extend out into the juxtaglomerular zone. Since normal glomerular capillary is essential to keep efficient ultrafiltration of the plasma, loss of mesangial cells due to pathologic conditions, such as glomerulonephritis and diabetic nephropathy, will impair kidney function (Prols et al., 1999). MSC are attractive candidates for kidney repair, because nephrons are of mesenchymal origin and stromal cells are of crucial importance for signalling, leading to differentiation of both nephrons and collecting ducts (Anglani et al., 2004). MSC can enhance recovery and protect against kidney failure (Togel et al., 2005; Qian et al., 2008; Cao et al., 2010). Bussolati et al. (2009) reported that MSC differentiate into tubular epithelial cells and repopulate the damaged tubules. Furthermore, Wong et al. (2008) found that MSC differentiated in vivo into kidney component cells, such as mesangial cells when injected into an injured kidney. However, these findings are only on MSC differentiation into kidney component cells under in vivo conditions. Thus, in this study, differentiation of MSC into mesangial cells was investigated under specific in vitro conditions.

Materials and methods

MSC and mesangial cells culture

Twenty millilitres of bone marrow aspirate were obtained from the iliac crest of an informed and consenting patient

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under the approval of the local hospital ethics committee. MSC were isolated based on Ficoll-Paque density-gradient centrifugation and adherence to a plastic surface. MSC were cultured in Dulbecco's modified Eagle's medium containing low glucose (DMEM-LG, Gibco), supplemented with 10% fetal bovine serum (FBS, Gibco), 100 U/mL penicillin, 100 mg/mL streptomycin, 250 ng/mL amphotericin B and 2 mM GlutaMAX (Gibco) (referred to as "complete culture medium" hereafter). The cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. After 3 days, non-adherent cells were discarded. Fresh complete culture medium was replaced every few days until the cells reached confluence. Adherent cells were collected with 0.25% trypsin and 1 mM EDTA (Gibco) and subcultured to expand the population of MSC. MSC were confirmed by immunophenotyping and their differentiation abilities (Wong et al., 2008).

Commercially available human mesangial primary cells (Lonza) were used, which were culture in complete culture medium.

MSC and mesangial cells culture preparation

One day prior to co-culture, MSC were harvested and replated in 6-well plate (1×10^4 cells per well). Replated MSC were allowed to adhere to the bottom. At the same time, mesangial cells were cultured in transwell and allowed to adhere on the culture insert (0.4 μm pore size) (1×10^4 cells per well).

Mesangial cells injury induction and co-culture with MSC

Injuries to the mesangial cells were induced by hydrogen peroxide. The optimum concentration of hydrogen peroxide was determined with lactose dehydrogenase assay. For co-culture, the mesangial cells were pretreated with 125 μM of hydrogen peroxide for 15 min, followed by washing twice with phosphate buffered saline before incubation in fresh complete culture medium for 1 h. Injured mesangial cells seeded on a cell culture insert were transferred to a six-well plates containing pure MSC. These two cell types were co-cultured for 7 days, separated by the membrane of the cell culture insert. Co-culture of MSC and uninjured mesangial cells (Control A), pure mesangial cells without co-culture (Control B) and pure MSC without co-culture (Control C) served as controls.

Immunophenotyping analysis

After 7 days, MSC on the bottom of the six-well plate were collected and characterised. They were stained for 20 min with 10 μL fluorescein isothiocyanate (FITC) or phycoerythrin (PE) conjugated anti-marker monoclonal antibodies. Tested markers included CD34, CD45, CD54, CD62E, CD73, CD90 and CD105 (Becton Dickinson). For isotype control, non-specific mouse Ig was substituted for the primary antibody. After incubation, the cells were washed with PBS and analysed by flow cytometery (FACScan, Becton Dickinson). For control purposes, cells from Control A, Control B and Control C were similarly characterised.

Contraction functional study

After 7 days, a portion of the MSC were seeded at 1×10^4 per well in a six-well plates. The culture medium was replaced with Hanks' balanced salt solution after 24 h and the cells stimulated with 10 nmol/L angiotensin II (Ang II) (Sigma). Images were taken at different times to record changes in morphology.

Results

Observation of MSC morphology after co-culture with injured mesangial cells

Prior to co-culture, MSC attached and showed a fibroblast-like appearance after being replated. Changes in the MSC to a mesangial cell-like morphology were seen at 7 days (Figure 1A), but not in the control (Figure 1B, Control A).

Immunophenotyping of MSC co-cultured with injured mesangial cells

Controls B and C consisting of pure mesangial cells and MSC populations, respectively, were immunophenotyped. The cells expressed CD73, CD90 and CD105, but not CD34 and CD45. MSC expressed CD54, but not CD62E, which is in direct contrast with mesangial cells expressing CD62E, but not CD54 (Figure 2).

MSC co-cultured with injured mesangial cell express CD73, CD 90 and CD105, but not CD 34 and CD45; they also expressed CD62E, but not CD54 (Figure 2). This is direct evidence that the cells had differentiated into mesangial cells as these changes were not seen in co-cultures with uninjured mesangial cells.

Contraction with Ang II

One characteristic feature of mesangial cells is that they contract in response to vasoactive peptides, for example Ang II, under in vitro conditions. Ang II induced contraction in MSC co-cultured with injured mesangial cells (Figure 3). Pure mesangial cells (Control B) and pure MSC population (Control C) were similarly treated with Ang II to serve as positive and negative controls, respectively.

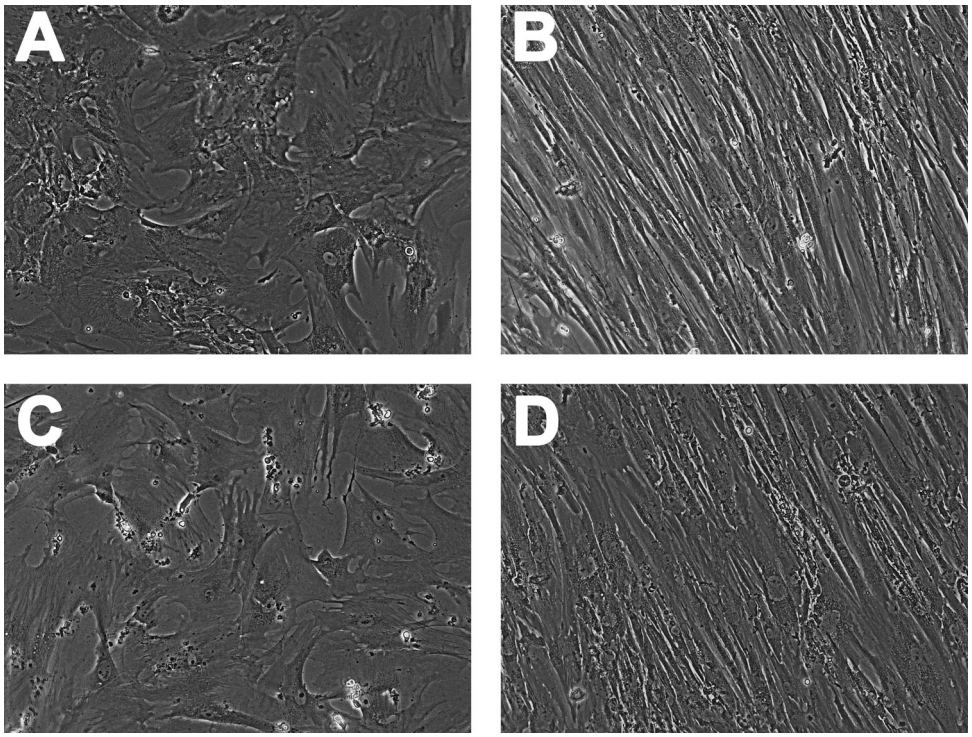

Figure 1 (A) After 7 days of co-culture with injured mesangial cells MSC morphology changed to a mesangial cell-like morphology. (B) MSC co-cultured with uninjured mesangial cell did not exhibit any change in morphology (Control A). (C) Morphology of pure mesangial cells without co-culture (Control B). (D) Morphology of pure MSC without co-culture (Control C).

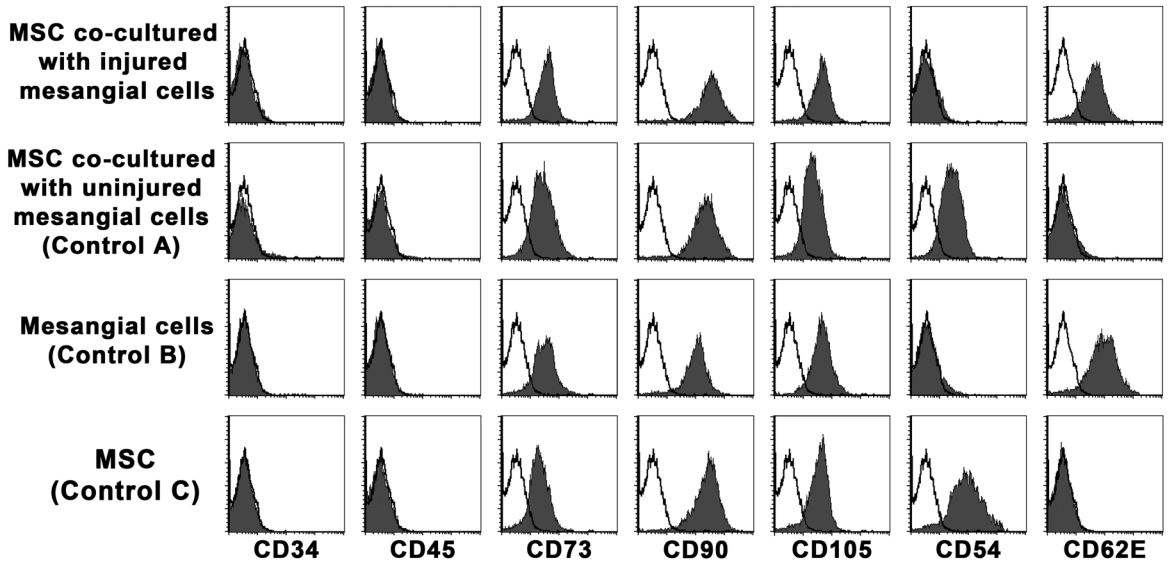

Figure 2 Immunophenotyping analysis: MSC co-cultured with injured mesangial cells expressed $CD54^{-}$ and $CD62E^{+}$. Meanwhile, MSC co-cultured with uninjured mesangial cells had the same expression profile as Control C of pure MSC population. The black lines represent isotype control and the gray histograms represent the CD markers tested. The *Y* axis of the histograms represented event count.

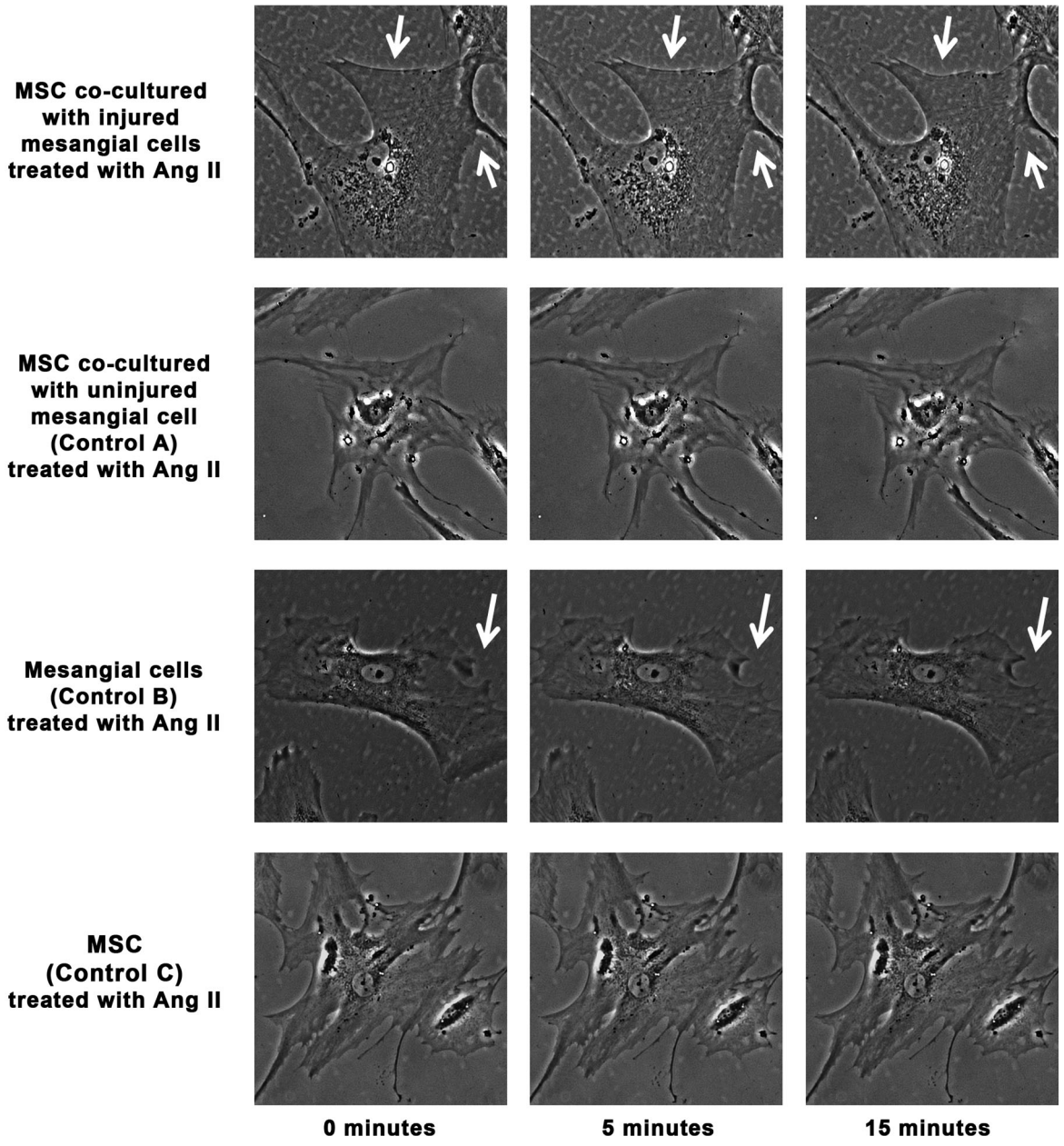

Figure 3 MSC co-cultured with injured mesangial cell were treated with Ang II; cell contraction was observed as indicated by white arrows. Control B of pure mesangial cells and Control C of pure MSC population treated with Ang II served as positive and negative controls, respectively.

Discussion

A protective role is seen with MSC in different types of kidney injury. The growing consensus is that this protective function acts predominantly through endocrine or paracrine factors released by MSC (Togel et al., 2005; Cao et al., 2010). The incidence of engraftment and differentiation of MSC into kidney component cells is variable (La Manna et al., 2011). Their ability to differentiate into kidney component cells, such as mesangial cells, as shown here opens up possibilities in adult stem cell differentiation. We successfully turned MSC into mesangial cells by co-culturing them with injured mesangial cells. The cells expressed CD62E, but not CD54, which is a direct reversal to their original CD54-positive and CD62E-negative status. Their morphology also resembled that of mesangial cells.

Expression of CD antigens in mesangial cells and MSC were almost identical. Mesangial cells expressed the same profile of CD antigens as MSC (CD14, CD34, CD73, CD90 and CD105), which are the antigens that identify MSC

(Dominici et al., 2006). However, two CD antigens were identified that could be used to differentiate mesangial cells from MSC. Akis and Madaio (2004) showed that mesangial cells expressed CD62E, but not CD54, whereas Jiao et al. (2011) found that MSC expressed the direct opposite. On this basis, these two antigens were chosen for further investigation.

We have shown MSC could differentiate into mesangial like cells when co-cultured with injured mesangial cells. Previous reports have indicated that MSC can differentiate into kidney tubular epithelial lineage when co-cultured with injured kidney tubular epithelial cells (Qian et al., 2008; Singaravelu and Padanilam, 2009), a phenomenon that might be due to molecular signals (growth factor and cytokines) released by the injured cells (Baer and Geiger, 2010). These molecular signals that could trigger MSC differentiation into mesangial like cells require further investigations.

Acknowledgments and funding

This study was supported by the Fundamental Research Grant Scheme (FGRS), Ministry of Higher Education, Malaysia.

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Received 2 October 2013; accepted 28 November 2013.
Final version published online 10 January 2014 .