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Surface markers distinguishing mesenchymal stem cells from fibroblasts

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Human mesenchymal stem cells (MSCs) are widely used for treatment of various diseases. Clinical applications require large quantities of MSCs, therefore these cells must be expanded in the culture system. It is believed that contamination of MSC cultures with fibroblasts may lead to the decrease of the stem cell differentiation potential. Moreover, such stem cell preparations are potentially unsafe to use for clinical applications since a few fibroblasts can become tumorigenic. Therefore, there is a need to separate MSCs from fibroblasts. However, studies show that MSCs and fibroblasts have much in common. These two types of cells share such properties as identical spindle-like morphology, plastic adherence and the same expression of most surface antigens. The aim of this review article is to analyze the literature on the similarities and differences between the MSCs and fibroblasts, particularly in the expression of cell surface markers in order to determine which could be used for quick separating of MSCs from fibroblasts. Interestingly, the results of recent studies suggest that the use of CD10, CD26, CD106, CD146 and ITGA11 could be helpful for the discrimination of MSCs from fibroblasts. Identification and elimination of fibroblasts from MSC cultures could improve the MSC yield and differentiation potential and also prevent possible tumor formation after MSC transplantation.

Key words: mesenchymal stem cells, fibroblasts, surface markers, senescence, tumorigenicity

INTRODUCTION

Human mesenchymal stem cells (MSCs) due to their regenerative and immunomodulatory properties are widely used for the treatment of bone and cartilage damage, cardiovascular, gastrointestinal, autoimmune, neurodegenerative diseases and cancer (1). It is shown that the use of MSCs in therapy is safe and can be effective (2). In 2006, the International Society for Cellular Therapy (ISCT) proposed the minimal criteria to define human MSCs. First, MSCs must be plastic-adherent when maintained in the standard culture conditions. Second,

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MSCs must express CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-DR surface molecules. Third, MSCs must differentiate into osteoblasts, adipocytes and chondroblasts in vitro (3). Fibroblasts are terminally differentiated stromal cells (4) that provide mechanical strength to tissues by producing extracellular matrix and play a critical role during tissue development, differentiation and repair in many organs (5).

It is believed that contamination of MSC cultures with fibroblasts may lead to the decrease of the stem cell differentiation potential because fibroblasts undergo senescence and eventually die. Moreover, such stem cell preparations are potentially unsafe to use for clinical applications since a few fibroblasts survive the crisis of senescence and can become tumorigenic (6). Therefore, there is a need to separate MSCs from fibroblasts. Although MSCs and fibroblasts have been well studied, differences between these two cell types are not fully understood (7). MSCs and fibroblasts share much in common and the current definition suggested by the ISCT is thus incapable of separating MSCs from fibroblasts (8). The aim of this review article is to analyze the literature on the similarities and differences between the MSCs and fibroblasts, particularly in the expression of cell surface markers in order to determine which could be used for quick separating of MSCs from fibroblasts.

Similarities between mesenchymal stem cells and fibroblasts

Mesenchymal stem cells (MSCs) and fibroblasts exhibit a similar spindle-like morphology. In addition to this, both types of cells adhere to plastic (7). Flow cytometry is a rapid method for separation of complex cell populations (9). However, MSCs and fibroblasts express the same surface markers. Alt E and colleagues (7) found that the expression of human adipose tissue-derived MSC surface markers CD44, CD90, CD105 was unspecific for these stem cells. Pure human embryonic lung fibroblasts were also positive for these markers. Both hematopoietic cell markers (CD14, CD45) and the endothelial cell marker (CD31) were absent in MSCs and fibroblasts. Halfon and colleagues (10) reported coincidental results. It was shown that human bone marrow MSC (BM-MSC) surface markers CD9, CD29, CD44, CD73, CD90, CD105, CD166 were also expressed on human dermal fibroblasts (10). Lorenz K and colleagues (11) showed similar expression patterns for CD14(–), CD29(+), CD31(–), CD34(–), CD44(+), CD45(–), CD71(+), CD73(+), CD90(+), CD105(+), CD133(–) and CD166(+) in human adipose tissue-derived stem cells and human dermal skin-derived fibroblasts. Cappellesso-Fleury S and colleagues (4) compared the expression of 25 surface markers of human BM-MSCs and human dermal fibroblasts. They found the similar expression patterns for 22 surface markers CD13(+), CD14(–), CD16(–), CD11a(–), CD33(–), CD34(–), CD43(–), CD45(–), CD49a(+), CD49b(+), CD54(+), CD86(–), CD90(+), CD105(+), CD117(–), CD146(+), CD164(+), CD166(+), CD138(variable), CD184(–), CD85k(–) and HLA-DR(–) in both types of cells.

Differences between mesenchymal stem cells and fibroblasts

Despite the fact that in the studies reviewed in this article the majority of the investigated cell surface markers were nonspecific, CD106, CD146 and ITGA11 have been identified as MSC-specific surface markers and CD10, CD26 as fibroblast-specific surface markers (10). ITGA11 is a member of integrins that binds to collagen and is involved in cell attachment, cell migration and collagen reorganization on mesenchymal non-muscle cells (12). Halfon S and colleagues (10) showed that only 16.7% of fibroblasts expressed ITGA11 on their surface compared with MSCs of early (51.4%) and late (28.6%) passages. CD106 (also known as vascular cell adhesion molecule-1 VCAM-1) is a member of the Ig superfamily which mediates leukocyte-endothelial cell adhesion and signal transduction during inflammation (13, 14). It was shown that CD106 protein was expressed only on MSCs but not on fibroblasts (10). CD146 (also known as melanoma cell adhesion molecule MCAM) is important for endothelial cell migration and angiogenesis (15). It was shown that only 4.83% of fibroblasts were CD146 positive compared with MSCs of early (91.7%) and late (79.8%) passages (10). Cappellesso-Fleury S and colleagues (4) reported different expression levels of CD10, CD106 and CD26 in comparison to 22 other markers. CD10 is a cell surface endopeptidase enzyme with neutral endopeptidase

activity and the ability to degrade a variety of biologically active compounds (16). CD26 is a cell surface glycoprotein known as dipeptidyl peptidase (DPP) IV and is involved in T lymphocyte activation (17). All fibroblasts were strongly positive for CD26 and CD10 whereas less than 35% of BM-MSCs expressed CD10 (range: 16–35%) and CD26 expression was variable (range 40–78%). By contrast, more than 70% of BM-MSCs expressed CD106 whereas all fibroblasts were negative (4). All studies of the MSC and fibroblast surface marker expression reviewed in this article are summarized in Table.

Other important specific features of MSCs in which these cells differ from fibroblasts are the colony-forming capacity and differentiation potential (7). Actually, the results of investigations of the fibroblast differentiation potential are controversial. Some authors state that fibroblasts do not differentiate into other types of cells (18). While the others show that fibroblasts do have the differentiation potential and even are potent immunoregulatory cells and functionally equivalent to mesenchymal stem cells (19). However, lately it has been supposed that such reported effects might be attributable to a great extent to the stem cell content within fibroblast preparations (7).

Table. Comparison of mesenchymal stem cell (MSC) and fibroblast surface marker expression

Cell surface marker	MSCs	Fibroblasts	References
CD9	+	+	(10)
CD10	±	+	(4)
CD13	+	+	(4)
CD26	**Variable**	+	(4)
CD29	+	+	(11), (10)
CD44	+	+	(7), (11), (10)
CD49a	+	+	(4)
CD49b	+	+	(4)
CD54	+	+	(4)
CD71	+	+	(11)
CD73	+	+	(7), (11), (10)
CD90	+	+	(4), (11), (10)
CD105	+	+	(4), (7), (11), (10)
CD106	+	–	(4), (10)
CD138	Variable	Variable	(4)
CD146	+	±	(4), (10)
CD164	+	+	(4)
CD166	+	+	(4), (11), (10)
ITGA11	+	–	(10)
CD11a	–	–	(4)
CD14	–	–	(7), (11), (4)
CD16	–	–	(4)
CD31	–	–	(7), (11)
CD33	–	–	(4)
CD34	–	–	(4), (11)
CD43	–	–	(4)
CD45	–	–	(4), (7), (11), (10)
CD86	–	–	(4)
CD117	–	–	(4)
CD133	–	–	(11)
CD184	–	–	(4)
CD85k	–	–	(4)
HLA-DR	–	–	(4)

Note: +: expression; –: no or low expression.

The need for separation of mesenchymal stem cells from fibroblasts

Clinical applications require large quantities of mesenchymal stem cells (MSCs). Expansion of cells in cultures is an attractive strategy because it makes it possible to administer more stem / progenitor cells than the patient can generate on his or her own. However, the differentiation potential of MSCs at later passages is often low. This phenomenon could be explained by contamination of MSC cultures with fibroblasts. It has been known for a long time that if fibroblasts from mouse embryos are cultured for prolonged periods, they undergo senescence followed by a "crisis" phase in which many of the cells die. The few cells that survive the "crisis" first become immortal in the culture and then, after further expansion, can become tumorigenic (6). Identification and elimination of fibroblasts from MSC cultures could improve the MSC yield and differentiation potential and also prevent tumor formation after MSC transplantation (10).

CONCLUSIONS

It is believed that the contamination of mesenchymal stem cell (MSC) cultures with fibroblasts can be unsafe. However, MSCs and fibroblasts share much in common and the current definition of MSCs suggested in 2006 by the International Society for Cellular Therapy is thus not capable of allowing us to discriminate between stem cells and fibroblasts any longer. Even then there were some thoughts that these criteria would probably require modification as new knowledge unfolded and that novel surface markers that might be identified in the future could lead to modifications of these criteria. The results of recent studies suggest that the use of CD10, CD26, CD106, CD146 and ITGA11 could be helpful for the discrimination of human bone marrow MSCs from human dermal fibroblasts. However, there is a need to confirm these surface markers by investigating their expression on MSCs and fibroblasts isolated from other human tissues. Eventually, such markers could be used for the quality control of MSC cultures after expansion, cryopreservation, gene transfection and other manipulations.

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MEZENCHIMINES KAMIENINES LĄSTELES NUO FIBROBLASTŲ ATSKIRIANTYS PAVIRŠIAUS ŽYMENYS

Santrauka

Žmogaus mezenchiminės kamieninės ląstelės (MKL) plačiai naudojamos įvairioms ligoms gydyti. Klinikinėje praktikoje reikalingi dideli MKL kiekiai, todėl šios ląstelės turi būti padaugintos kultūrose. Manoma, kad MKL kultūrose esantys fibroblastai yra susiję su silpnėjančiu MKL diferenciacijos potencialu. Tokius kamieninių ląstelių preparatus pavojinga naudoti terapijoje, nes dalis fibroblastų gali supiktybėti, todėl fibroblastus iš MKL kultūrų reikia pašalinti. Mokslinių tyrimų rezultatai rodo, kad MKL ir fibroblastai yra labai panašūs. Šioms abiejų tipų ląstelėms būdinga identiška verpstės formos morfologija, adhezija prie plastikinių paviršių ir panaši daugumos paviršiaus žymenų raiška. Šio straipsnio tikslas – apžvelgti literatūrą apie MKL ir fibroblastų panašumus ir skirtumus, daugiausia dėmesio skiriant tyrimams, susijusiems su ląstelių paviršiaus žymenų, pagal kuriuos būtų galima greitai atskirti MKL nuo fibroblastų, raiška. Fibroblastų atpažinimas ir pašalinimas iš MKL kultūrų turėtų lemti didesnius auginamų MKL kiekius, stipresnį šių kamieninių ląstelių diferenciacijos potencialą ir padėtų išvengti galimo navikų formavimosi po MKL transplantacijos. Naujų tyrimų rezultatai rodo, kad ląstelių paviršiaus žymenys CD10, CD26, CD106, CD146 ir ITGA11 gali būti naudingi siekiant atskirti MKL nuo fibroblastų.

Raktažodžiai: mezenchiminės kamieninės ląstelės, fibroblastai, paviršiaus žymenys, senėjimas, navikų formavimasis