

Original Research

## Differences of cell surface marker expression between bone marrow- and kidney-derived murine mesenchymal stromal cells and fibroblasts

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**Abstract:** Mesenchymal stromal cells (MSC) are undifferentiated, multipotent adult cells with regenerative properties. They are particularly relevant for therapeutic approaches due to the simplicity of their isolation and cultivation. Since MSC show an expression pattern of cell surface marker, which is almost identical to fibroblasts, many attempts have been made to address the similarities and differences between MSC and fibroblasts. In this study we aimed to isolate murine MSC from bone marrow (BM) and kidney to characterize them in comparison to fibroblasts. Cells were isolated from murine kidney, BM and abdominal skin by plastic adherence and subsequently characterized by analysing their capability to build colony-forming unit-fibroblasts (CFU-F), their morphology, their proliferation, expression of telomerase activity and cell surface antigens as well as their differentiation capacity. Plastic adherent cells from the 3 mouse tissues showed similar morphology, proliferation profiles and CFU-F building capacities. However, while MSC from BM and kidney differentiated into the adipogenic, chondrogenic and osteogenic direction, fibroblasts were not able to do so efficiently. In addition, a tendency for lower expression of telomerase was found in the fibroblast population. Proliferating cells from kidney and BM expressed the MSC-specific cell surface markers CD105 and Sca-1 on a significantly higher and CD117 on a significantly lower level compared to fibroblasts and were thereby distinguishable from fibroblasts. Furthermore, we found that certain CD markers were specifically expressed on a higher level, either in BM-derived cells or fibroblasts. This study demonstrates that murine MSC isolated from different organs express certain specific markers, which enable their discrimination.

**Key words:** Mesenchymal stromal cells, fibroblasts, regeneration, cell surface markers, flow cytometry, multipotent cells, cell differentiation.

### Introduction

MSC are multipotent cells, which can be isolated from various organs (1) and have been implicated in tissue maintenance and repair. Recent studies suggest possible paracrine and endocrine effects of MSC including mitogenic, angiogenic antiapoptotic and anti-inflammatory influences (2). The simplicity of isolation and cultivation of MSC as well as their regenerative properties make these cells very attractive as sources for therapeutic applications in regenerative medicine. Since their first description by Friedenstein *et al.* (3) intensive investigations have been carried out to characterize their properties and functionality *in vitro* and *in vivo*. To date, a huge variety of cultivation and isolation conditions have been published. The different conditions may have a strong influence on the cells characteristics. Furthermore, specific markers are missing and the described cells seem to represent rather heterogeneous cell populations (4). However, the combination of several criteria is helpful to characterize MSC: plastic adherence when maintained in standard cultures *in vitro*, differentiation into osteoblasts, adipocytes and chondroblasts *in vitro* and expression of a set of cell surface markers (5). Unfortunately, none of the cell surface markers are solely specific to MSCs. There is a consensus that human MSCs should be positive for CD44, CD73, CD90 and CD105. Murine MSCs (mMSCs) are particularly difficult to characterize due to their low proliferative activity *in vitro* and substantial contamination of cultures by the

cells of haematopoietic origin (6). Furthermore, experience with pluripotent cells shows that expression of surface epitopes in mouse cells does not necessarily coincide with the human counterpart (7). Indeed mMSCs vary in the frequency of the CD73-, CD90- and CD105-positive cells, but express stem cell antigen-1 (Sca-1) and CD44 (8, 9). Also variations in immunophenotype due to the site of origin have been described, suggesting that certain properties are at least partially organ-specific.

Recently, the similarities and differences between MSC and fibroblasts have been in the focus of interest. Several lines of evidence indicate that cells considered to be fibroblasts not only express surface markers almost identical to MSCs, but could also differentiate into tissue of the three germ layers (10, 11). Furthermore, contamination of MSC cultures with fibroblasts may lead to a decrease of stemness properties since fibroblasts undergo senescence and eventually die. Another concern is that surviving fibroblasts may become tumorigenic (12).

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We performed an extensive study of surface markers using murine MSCs from different tissues and fibroblasts from mouse skin. Our MSCs from kidney and BM both expressed certain cell surface markers on a significantly higher level in comparison to fibroblasts. Some CD markers were specifically expressed in either BM-derived cells or fibroblasts.

## Materials and Methods

### Experimental animals

Male adult NMRI-mice obtained from Charles River Laboratories (Kisslegg, Germany) were housed in a temperature-controlled environment with a 12-hour light/dark cycle and had free access to food and water. The experimental studies were performed in accordance with German law and approved by the State Agency for Agriculture, Environment and Rural Areas (LLUR), Germany (V312-72241.122-4).

### Isolation of MSC

Mice were sacrificed by cervical dislocation and the femurs, tibias and kidneys were removed. The possibility that MSC cultures were partially or entirely derived from circulating blood was excluded by intravascular perfusion of the animals before organ collection. Different strategies were used to isolate the cells (Figure 1). BM-derived cells were collected by flushing femurs and tibias with cultivation medium. The kidneys were released from the surrounding tissue, cleaned, minced and incubated in a collagenase/dispase-solution for 40 min at 37 °C and 75 rpm. The tissue/cell suspension was then filtrated through a sterile sieve (appr. 500 µm pore size). The cell suspension was then centrifuged and the collected cells were disseminated into culture medium.

Abdominal skin was shaved, cut into 5x5 mm pieces and put onto the dry surface of our cultivation flasks. After 4 hours the pieces of skin were surrounded by medium. They were not removed until we could detect outgrowing fibroblasts under the microscope (mostly after 3 weeks of cultivation).

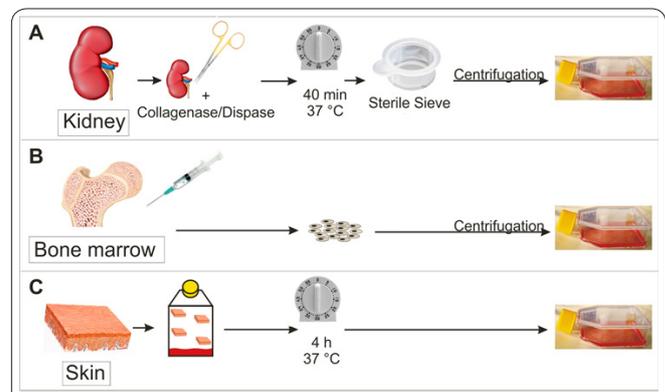
### Cultivation of MSC

The isolated cells were disseminated in basal medium consisting of Dulbecco's modified Eagle medium (DMEM, GIBCO-BRL, Grand Island, NY, USA) containing 10% fetal bovine serum (Thermo Fisher Scientific, Waltham, USA), 1% MEM non-essential amino acids (Invitrogen, Karlsruhe, DE), 1% l-glutamin (PAA, Pasching, Austria), 1% penicillin/streptomycin (PAA, Pasching, AT), 1% sodium-pyruvate (PAA, Pasching, AT) and 300 µM vitamin C (sterile, Sigma, München, DE).

After 24 hours non-adherent cells and debris were discarded and fresh medium was added to the adherent cells. Cultures were maintained at 37 °C and 5% CO<sub>2</sub>. Cell growth was controlled under the microscope and the passaging took place at a cell confluence of 70-80%. Before this degree of confluence was achieved, and not later than 7 days after isolation, colony-forming unit-fibroblasts (CFU-F) were counted under the microscope.

### Proliferation analysis

We analyzed the cell proliferation rates after 24 h, 48



**Figure 1. Preparation of plastic adherent cells.** After withdrawal organs were cleaned from neighbored tissue. Kidney cell cultures were established by mincing and enzymatic treatment of the tissue using collagenase (A). In the case of BM the bone cavity was opened and BM was extracted by flushing with sodium chloride solution (B). Fibroblasts were isolated from migrating cells by cultivation of belly skin pieces (C).

h and 72 h of cultivation after incorporation of 5'-Bromo-2'-deoxy-Uridin (BrdU) by immunostaining using an anti-BrdU mAb, clone BMG 6H8 (Roche Diagnostics, Mannheim, Germany) and a fluorescein isothiocyanate (FITC, Dianova, Hamburg, Germany; 1:200) labelled anti-mouse IgG secondary mAb as described in 4.9 well as DAPI labelling. The ratio BrdU<sup>+</sup>/DAPI<sup>+</sup>-cells to BrdU<sup>-</sup>/DAPI<sup>+</sup>-cells was defined under the microscope (20x).

### Telomerase activity analysis

Telomerase activity was determined using the *TeloTAGGG Telomerase PCR ELISA Kit* (Roche Diagnostics, Mannheim, Germany) following the manufacturer protocol.

### Flow cytometry

EDTA/trypsin-(0.25%) treated MSC (passage 4 - 5) and fibroblasts were washed twice with FACS buffer [PBS, 1% BSA and 0.1 NaN<sub>3</sub> in phosphate-buffered saline (PBS)], adjusted to approximately 1 x 10<sup>6</sup> cells and subsequently stained. A 100 µl cell suspension was incubated with either 20 µl phycoerythrin (PE) - conjugated monoclonal antibodies (mAbs) or 10 µl non-conjugated mAb and a secondary goat anti-mouse IgG1-PE at 4 °C for 15 min. All mAbs against the mouse antigens CD29, CD34, CD44, CD45, CD49a, CD49d, CD54, CD73, CD81, CD105, CD106, CD117, CD133, CD140b, CD166, Sca-1, and CD292 were purchased from Becton Dickinson (Heidelberg, Germany). MSC were analyzed on an Epics XL flow cytometer using the System II software (Beckman Coulter, Krefeld, Germany).

### Cell differentiation

Cells were differentiated into the adipogenic, chondrogenic and osteogenic direction using induction media as described previously (13-15). Briefly, to induce adipogenic differentiation, cells in monolayer culture were treated with adipogenic induction medium, which consisted of basal medium supplemented with 0.5 mM 3-isobutyl-1-methylxanthine (IBMX Sigma, München, Germany), 1 µM dexamethasone (Merk, Darmstadt, Germany), 200 µM indomethacin (Sigma, München,

Germany) and 2  $\mu$ M insulin (Sigma, München, Germany). Following a four-day induction period, the adipogenic induction medium was replaced with adipogenic maintenance medium consisting of basal medium supplemented with 2  $\mu$ M insulin for three days. This cycle was repeated three times, followed by a four-day period of adipogenic maintenance culture.

To induce chondrogenic differentiation, cells were cultivated as micromass bodies, by brief centrifugation of  $2 \times 10^5$  cells in 0.5 ml chondrogenic induction medium, which consisted of basal medium supplemented with 0.1  $\mu$ M dexamethasone (Merk, Darmstadt, Germany), 300  $\mu$ M ascorbic acid (Sigma, München, Germany), 1 mM L-proline (Sigma, München, Germany), 10 ng/ml transforming growth factor (TGF)  $\beta$ 3 (R&D, Wiesbaden, Germany) and 1% ITS premix (Becton Dickinson, Heidelberg, Germany: 6.25  $\mu$ g/ml insulin; 6.25  $\mu$ g/ml transferrin; 6.25  $\mu$ g/ml selenic acid; 1.25 mg/ml bovine serum albumin; 5.35 mg/ml linoleic acid).

For osteogenic differentiation, cells in monolayer culture were cultivated in osteogenic medium consisting of basal medium supplemented with 0.1  $\mu$ M dexamethasone (Merk, Darmstadt, Germany), 10 mM  $\beta$ -glycerolphosphate (Sigma, München, Germany) and 300  $\mu$ M ascorbic acid (Sigma, München, Germany).

### Histochemical staining

Lipid accumulation during adipogenic differentiation was demonstrated by Sudan III staining. Cells were washed with PBS followed by staining with a 0.2% solution of Sudan III (Sigma, München, Germany) in 70% ethanol. Alkaline Phosphatase (AP) activity of cells was demonstrated by AP staining (125  $\mu$ l FRV-alkaline solution (Sigma, München, Germany), 125  $\mu$ l sodium nitrite solution (Sigma, München, Germany), 125  $\mu$ l naphthol AS-BI alkaline solution (Sigma, München, Germany), 5.63 ml aqua dest.) after fixation of cells.

### Fluorescence immunostaining

Micro mass bodies were embedded in Tissue-Tek O.C.T. (Sakura Finetechnical, Tokyo, Japan), frozen at  $-80^\circ\text{C}$  and cryosectioned (10  $\mu$ m). Sections were rinsed three times with PBS, fixed for 5 min with pre-cooled ( $-20^\circ\text{C}$ ) methanol-acetone at  $4^\circ\text{C}$ , washed four times with PBS and incubated at room temperature for 30 min with 7.5% bovine serum albumin. Specimens were then incubated for 1 hour with the primary antibody II-II-6B3, diluted 1:20 with PBS in a humidified chamber at  $37^\circ\text{C}$ . The antibody was obtained from the Developmental Studies Hybridoma Bank (University of Iowa, Iowa City, IA, USA). After rinsing four times with PBS, slides were incubated for 1 hour at  $37^\circ\text{C}$  with fluorescein isothiocyanate labelled anti-mouse IgG (FITC, Dianova, Hamburg, Germany; 1:200). Slides were washed four times in PBS and briefly washed in distilled water. After immunostaining, the specimens were embedded in Vectashield mounting medium (Vector, Burlingame, CA, USA) and analyzed with the fluorescence microscope Axioskop (ZEISS, Oberkochen, Germany). Negative controls were performed using the secondary antibody alone.

### RT-PCR analysis

Differentiated cells or micro mass bodies were col-

lected at different time points, washed twice with PBS, and total RNA was isolated using a standardized RNA Isolation Kit (Macherey & Nagel, Düren, Germany). The RNA concentrations were determined by measuring the absorbance at 260 and 280 nm. Samples of 500 ng RNA were reverse transcribed using oligo-dT primer and Superscript II reverse transcriptase following the manufacturer's recommendations (Invitrogen, Paisley, U.K.). Aliquots of 1  $\mu$ l from the reverse transcriptase reactions were used for amplification of transcripts using primers specific for the analyzed genes and Taq polymerase according to the manufacturer's instructions (Fermentas, St. Leon, Germany). Reverse transcriptase reactions were denatured for 2 min at  $95^\circ\text{C}$ , followed by amplification for 30–40 cycles of 40 sec denaturation at  $95^\circ\text{C}$ , 40 sec annealing at the primer-specific temperature and 50 sec elongation at  $72^\circ\text{C}$ . Primers specific for the following genes were used (sequence, annealing temperature as well as size are given in parentheses): PPAR $\gamma$  (5'-GCCTAAGTTTGAGTTTGCTGTG-3', 5'-TGTCATCTTCTGGAGCACCTT-3',  $58^\circ\text{C}$ , 226 bp), aP2 (5'-ATGCCTTTGTGGGAACCT-3', 5'-GCT-TGTCACCATCTCGTTTT-3',  $58^\circ\text{C}$ , 333 bp), adipsin (5'-CTGACAGCCTTGAGGACGA-3', 5'-AGAGCC-CCACGTAACCACA-3',  $58^\circ\text{C}$ , 356 bp), osteopontin (5'-TCACTCCAATCGTCCCTACA-3' 5'-TGCTCA-AGTCTGTGTGTTCC-3',  $58^\circ\text{C}$ , 289 bp), aggrecan (5'-CAGAAACAACCATGTCCCTGA-3', 5'-TGT-GACTGCTGCTCCCGAC-3',  $59^\circ\text{C}$ , 196 bp), runx2 (5'-CCAAGAAGGCACAGACAGAA-3', 5'-CAG-ATAGGAGGGTAAGACTGG-3',  $61^\circ\text{C}$ , 243 bp), GAPDH (5'-GGAAGGGCTCATGACCACA-3', 5'-CCGTTTCAGCTCTGGGATGAC-3',  $58^\circ\text{C}$ , 164 bp). Electrophoretic separation of PCR products was carried out on 2% agarose gels (2% (w/v) agarose (Roth, Karlsruhe, Germany), 0.7 ng/ml ethidium bromide (Roth, Karlsruhe, Germany). Distilled water and total RNA from a 19 d old mouse embryo were included respectively as negative and positive controls.

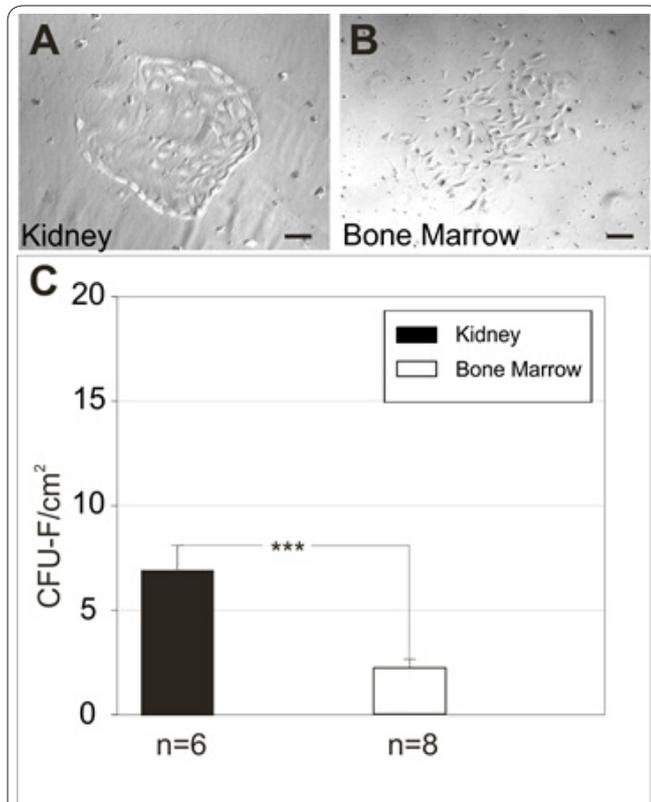
### Statistical analysis

All values are expressed as mean  $\pm$  SEM. Data analysis was carried out using the Sigma Plot 5.0 software (JANDEL, Corte Madeira, CA) and Prism 5 software (GraphPad Software, La Jolla, USA). One way Anova and Bonferroni post hoc test were performed for evaluation of statistical significance.

### Results

#### Successful isolation of plastic adherent cells from kidney and BM

Plastic adherent cells from the analyzed murine organs were isolated using standard procedures. Although the number of CFU-Fs was significantly different between kidney and BM (Figure 2), the colonies of different origin showed only subtle morphological differences (Figure 3). Interestingly, we found differences in the morphological appearance among the plastic adherent cells in cultures of the same origin after isolation. These different cell types were also detectable in higher passages, but disappeared as soon as confluency was achieved. Only the skin-derived fibroblasts showed a homogeneous morphology from the beginning, with



**Figure 2. Colony forming unit fibroblasts (CFU-F) from kidney and BM.** Cells isolated from kidney (n=6), and bone marrow (n=8) formed CFU-F (A, B). The number was determined and found to be significantly different per cm<sup>2</sup> tissue culture dish (C). \*\*\* =  $p < 0.001$ ;  $\pm$  SE.

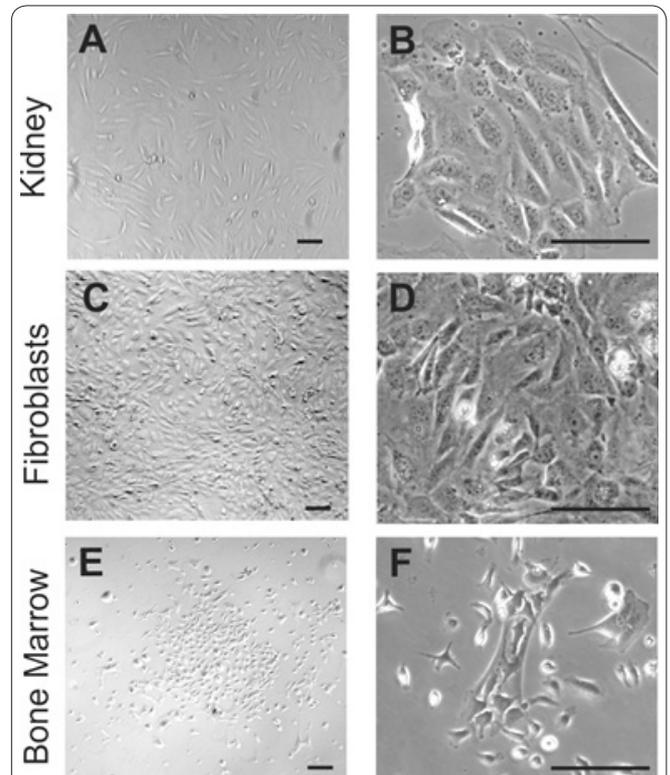
spindle shaped cells and a narrow cytoplasm seam (Figure 3C, D).

#### Proliferation profile and telomerase activity

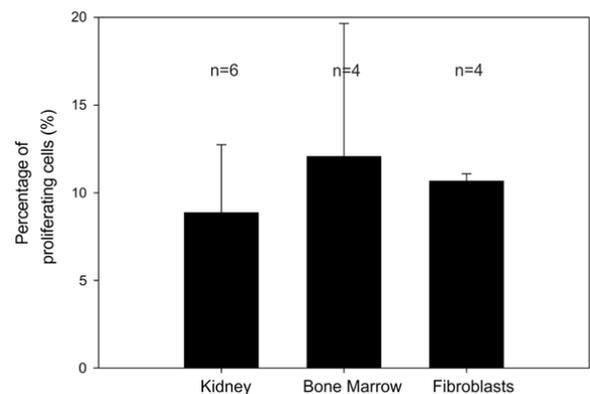
Proliferation rates of BM-, kidney- and skin-derived cells did not show any significant differences (Figure 4). Also, telomerase activity could be detected in every cell population (Figure 5). Fibroblasts showed a tendency of lower activity compared to the other cells although the differences were not statistically significant.

#### Plastic adherent cells from kidney, BM and abdominal skin can be discriminated by their surface markers

The analysis of cell surface markers indicated that the MSC populations originating from the two tissues have a similar immunophenotype, but can at least be distinguished from the fibroblasts (Figure 6). All cell populations showed similar percentages positive for CD29 (integrin  $\beta$ -1), CD44 (hyaluronate receptor), CD54 (intercellular adhesion molecule-1), CD73 (5'-nucleotidase), CD81 (member of tetraspanin family), CD140b (beta-type platelet-derived growth factor receptor) and CD166 (activated leukocyte cell adhesion molecule [ALCAM]). However, for some markers the number of positive cells showed remarkable variability indicating heterogeneous cell populations. The haematopoietic progenitor marker CD34 was only detectable in a small amount of cells from BM (in average 29% of all cells), while the hematopoietic marker CD45 was not detected in any cell isolate. For some markers a higher number of positive cells was detected in the skin-derived fibroblast populations. This was most obvious

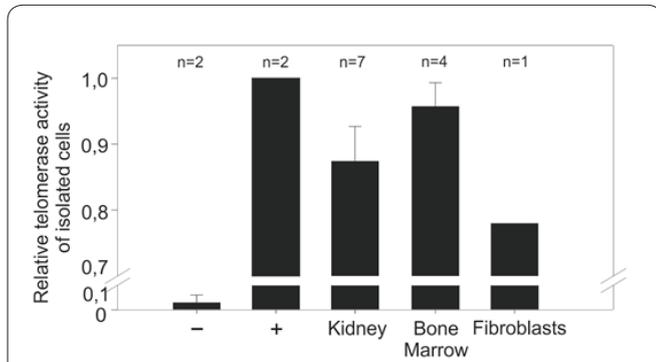


**Figure 3. Morphology of cells isolated from different murine tissues.** Morphological appearance of cells isolated from kidney (A, B), skin (C, D) and BM (E, F). Only minor morphologic varieties were observed. Representative pictures are shown. Bars = 150  $\mu$ m.

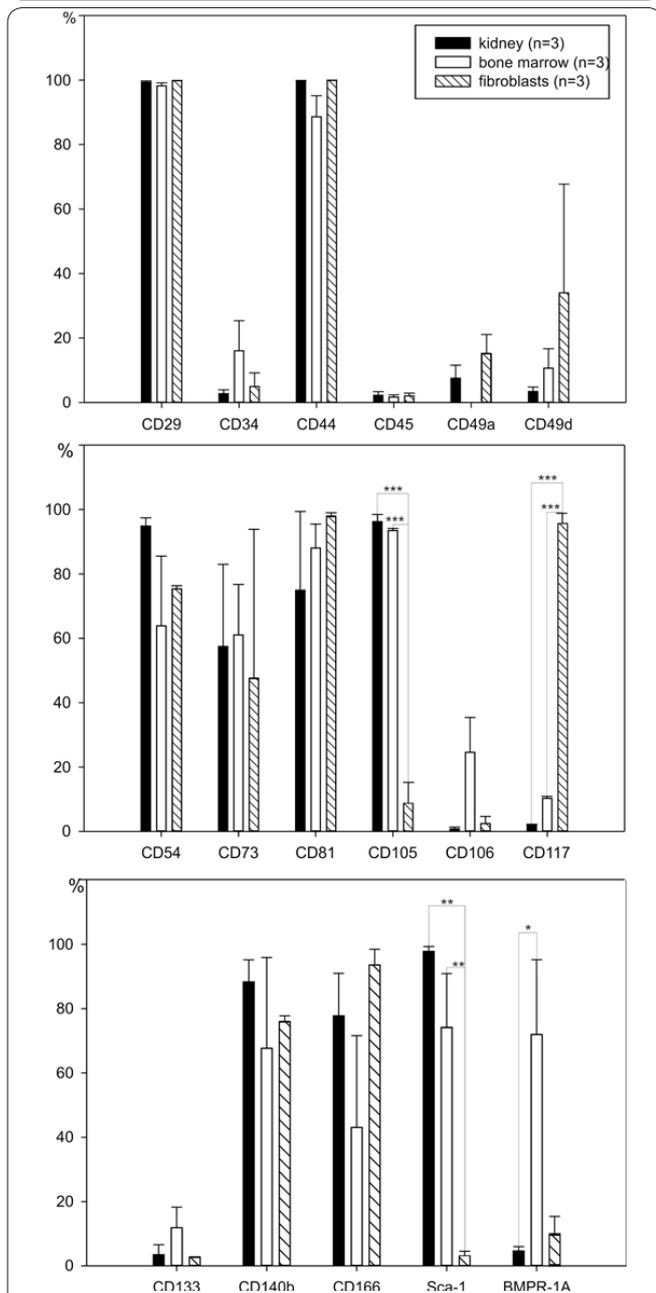


**Figure 4. Cell proliferation rates of cells isolated from different murine tissues.** Cells isolated from different tissues were cultivated for 24 h and proliferation was analysed by immunofluorescence and cell counting after incorporation of BrdU and labelling with DAPI. No significant differences were found between cells from kidney, BM and skin. (\* =  $p < 0.05$ ;  $\pm$  SE).

in the case of CD117 (c-kit, stem cell factor receptor), which was expressed by a significantly higher number of cells in the fibroblast preparation in comparison to BM- and kidney-derived cells. Also CD49d, an integrin receptor of mainly mesenchymal tissue, was expressed by a low amount of BM and kidney cells but more cells were positive in the skin-derived cell population. For at least two markers, Sca-1 (stem cell antigen-1) and CD105 (endoglin) a significantly higher amount of cells was found in both the BM- and kidney-derived populations compared to those isolated from skin. Finally, for two markers, CD292 (BMP receptor 1A) and CD106, a higher number of positive cells was detected only in the



**Figure 5. Telomerase activity of cells isolated from different murine tissues.** Using the ELISA technique, telomerase activity was determined in cells from different origin. A tendency for lower activity was found in fibroblasts in comparison to the MSCs. - = telomerase negative controls, + = telomerase positive controls.

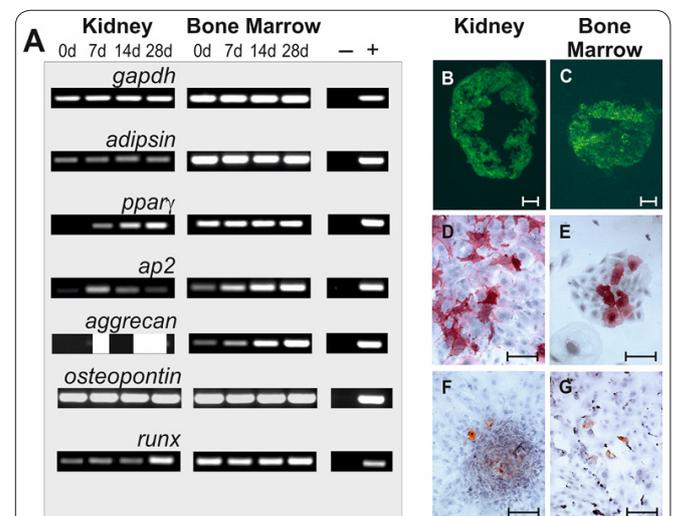


**Figure 6. Flow cytometric analysis of cells isolated from different murine tissues.** Cells were detached with Trypsin-EDTA, labelled with a mAb conjugated with phycoerythrin and analyzed for expression of different CD marker molecules as indicated. Data from three independent experiments are shown. (\* =  $p < 0.05$ ; \*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.001$ ;  $\pm$  SE).

BM-derived population. This was most obvious in the case of CD292. While the detection rate was low in cells from kidney and skin, BM-MSCs contained about 70% CD292<sup>+</sup> cells. CD106 (vascular cell adhesion molecule [VCAM]-1), which is functionally associated with hematopoiesis, was exclusively expressed in BM-derived cells, whereas expression in cells from kidney and skin was negligible. Only one marker molecule, CD49a, was expressed in kidney-derived cells and fibroblasts but not in BM-derived cells. In summary, we found specific differences in the expression of the CD markers for BM-derived cells and fibroblasts and at least two markers, CD105 and Sca-1, which were exclusively upregulated in both MSC populations in comparison to the fibroblasts.

**Plastic adherent cells from kidney and BM differentiate into adipogenic, chondrogenic and osteogenic cells**

Successful differentiation of plastic adherent cells from kidney and BM, cultivated in specific induction media, either as monolayer or as micro mass bodies, was demonstrated by analysis of tissue-specific gene expression using RT-PCR, by immunostaining and histochemical staining (Figure 7). During cultivation at days 7, 14 and 28 in adipogenic induction media, the adipogenic marker genes *adipsin*, *ppary* and *ap2* were found to be expressed in kidney- and BM-derived cells, although on a higher level in BM probes (Figure 7A). The same was true for the expression of aggrecan in mi-



**Figure 7. Differentiation of MSC derived from murine BM and kidney.** MSCs from BM and kidney were differentiated into the adipogenic, osteogenic and chondrogenic direction using specific induction media for cultivation. After different time points during differentiation, more specifically on the day of induction (0d) and on days 7, 14 and 28 after induction (7d, 14d, 28d), total RNA was isolated, reverse transcribed and amplified by PCR using primers specific for adipogenic (*adipsin*, *ppary*, *ap2*), chondrogenic (*aggrecan*) and osteogenic (*osteopontin*, *runx2*) genes (A). Controls used were water (-) and total RNA from a 19 day old mouse embryo (+). BM: n=3; kidney: n=4. Differentiated chondrogenic cells expressed collagen type II as demonstrated by immunostaining of micromass body sections (B, C), osteogenic cells expressed alkaline phosphatase as shown by histochemical staining (D, E) and adipogenic cells were positive for Sudan III (F, G) as shown by histochemical staining. Bars = 100  $\mu$ m.

cro mass bodies from kidney- and BM-derived cells cultivated in chondrogenic induction media and the bone markers osteopontin and runx2 in cultures from kidney- and BM-derived cells with osteogenic induction media. Immunostaining demonstrates that the cartilage matrix molecule collagen type II is expressed in kidney- and BM-derived micromass bodies cultivated in chondrogenic induction media (Figure 7B, C), confirming the chondrogenic differentiation. Staining of kidney- and BM-derived cells for alkaline phosphatase after cultivation in osteogenic induction media (Figure 7D, E) confirmed the differentiation of osteocytes and staining of lipid droplets in kidney- and BM-derived cells after cultivation in adipogenic induction media (Fig. 7F, G), confirmed the differentiation of adipocytes. Taken together, these results demonstrate that the analyzed murine cells from kidney and BM were able to differentiate into the adipogenic, chondrogenic and osteogenic lineage.

## Discussion

Since MSC can be isolated and cultivated easily and have broad differentiation potential as well as immunologic characteristics, they are a promising source for tissue engineering and gene therapy. MSC and fibroblasts exhibit similar morphologies, but have different functional properties (16). However, evidence exists that fibroblasts and MSC are similar cell types (10, 11). This necessitates strategies to distinguish MSC from fibroblasts.

In our study we characterized plastic adherent murine cells from kidney and BM as potential tissue sources of MSCs. Our results confirm the existence of multipotent MSC in murine tissues other than BM, such as kidney, and reflect previous reports showing the existence of MSC in many adult organs (1). Furthermore our findings are congruent to previous study results, which describe that MSC isolation by classical protocols yields a heterogeneous population of progenitor cells (17). Although we used identical isolation and cultivation methods, cell cultures showed a high variability regarding morphology and proliferation, even among isolates from the same source. One reason for this heterogeneity could be our simple MSC isolation protocol based on the plastic adherence property. The amount of multipotent MSC could also vary due to physiological circumstances and irregular anatomic distribution. It may be possible that tissues contain already differentiated MSC-derived precursor cells and differentiated cell types in addition to the undifferentiated MSCs. This hypothesis is supported by the findings of several studies (18, 19). Furthermore, MSC may be able to change their fate during culture due to their plastic nature (20). The application of specific induction media following classical differentiation protocols resulted in an efficient differentiation of the cell isolates from BM and kidney into the adipogenic, chondrogenic and osteogenic lineage. This was not the case for the cells from skin, indicating that these cells are fibroblasts and not MSC and confirming that *in vitro* differentiation is a reliable test to discriminate MSC and fibroblasts.

It has been demonstrated that MSC express a panel of surface marker molecules (5, 21, 22). In accordance with these observations we show that kidney and BM

cell isolates were negative for CD34 and CD45, but expressed CD29, CD44, CD54, CD73, CD81, CD140b and CD166. However, we observed that the expression of surface marker molecules differed among plastic adherent cells from the analyzed murine tissues. In particular, we found almost exclusive expression of the BMP receptor 1A (CD292) on BM-derived cells. Furthermore, CD106<sup>+</sup> cells were only found in BM preparations. If we consider the phenotype variability of MSC these differences in the expression of surface markers may be regarded as a specialization of MSC due to their environmental influences. This means that renal MSCs may be discriminated from MSCs of other origin. But renal MSCs may also be able to show different marker expression patterns if cultivated under different conditions. Further studies are required to compare for example renal multipotent MSCs isolated by different methods and cultivated under different conditions.

The most notable findings of our study were that marker CD117 was exclusively expressed by fibroblasts and markers CD105 and Sca-1 were not expressed by fibroblasts at all. These marker molecules may therefore be used to discriminate fibroblasts from MSC of other origins. Another field of further investigation should be the influence of certain marker molecules, especially Sca-1, on cell fate. Although it is the most common marker used to enrich adult murine hematopoietic stem cells, little is known about the biochemical function of Sca-1 (23). It is also unclear if Sca-1 has a human ortholog.

In summary, our study suggests that in particular markers CD105, CD117, CD292 and Sca-1 may be helpful for the discrimination of murine MSC and fibroblasts. However, it is unclear how the expression of these surface markers is influenced by longer cultivation periods. Further studies are needed to establish such markers for the use in quality control of the MSC cultures after expansion and cryoconservation.

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