

DOI 10.2478/pjvs-2013-0084

Review

# Characterization of mesenchymal stem cells and their application in experimental embryology

J. Opiela<sup>1</sup>, M. Samiec<sup>2</sup>

National Research Institute of Animal Production, Department of Biotechnology of Animal Reproduction, Krakowska 1, 32-083 Balice n. Kraków, Poland

## Abstract

The efficiency of somatic cell cloning (somatic cell nuclear transfer; SCNT) as well as *in vitro* fertilization/*in vitro* embryo production (IVF/IVP) in mammals stay at relatively same level for over a decade. Despite plenty of different approaches none satisfactory break-through took place. In this article, we briefly summarize the implementation of mesenchymal stem cells (MSCs) for experimental embryology. The advantages of using MSCs as nuclear donors in somatic cell cloning and *in vitro* embryo culture are described. The description of results obtained with these cells in mammalian embryo genomic engineering is presented.

**Key words:** mesenchymal stem cell, *in vitro* culture, somatic cell cloning, embryo

## Characteristics of mesenchymal stem cells and their potential for differentiation

Mesenchymal stem cells (MSCs), also termed multipotent marrow stromal cells or mesenchymal stromal cells are a heterogeneous population of plastic-adherent, fibroblast-like cells, which in culture are able to self-renew and differentiate into bone, adipose and cartilage tissue (Horwitz et al. 2005). The MSCs have traditionally been isolated from the bone marrow (BM) but isolation of cells with MSC characteristics from a variety of tissues including cord blood, peripheral blood, fetal liver and lung, adipose tissue, skeletal muscle, amniotic fluid and the circulatory system is applied. The MSCs are rare in the BM (occur-

ring at an estimated frequency of 1 in  $1 \times 10^5$  cells in the human bone marrow) but can be isolated by selecting the adherent, spindle-shaped cells that expand from mononuclear cells in humans, rodents and pigs (Caplan 2007). Different cell types of predominantly mesodermal origin could be identified in the adherent fraction of bone marrow mononuclear cells including embryonic-like stem cells, lineage committed progenitors as well as mature cells such as osteoblasts and fibroblasts (Jiang et al. 2002, Ratajczak et al. 2004, 2008). MSCs may also generate mature cells typically arisen from endoderm or ectoderm (Oh et al. 2007, 2008, Tondreau et al. 2008) suggesting that *in vitro* cultures of bone marrow-derived MSCs may represent a mixture of phenotypically, functionally and bio-

---

<sup>1</sup> Correspondence to: J. Opiela, e-mail: jolanta.opiela@izoo.krakow.pl

<sup>2</sup> The authors contributed equally to the preparation of the manuscript

chemically different cells (Phinney 2007, Jones and McGonagle 2008). The amount of primitive stem cells in these cultures is rare and varies depending on the method of cell isolation or culture conditions and the donor's age (Wagner and Ho 2007, Ho et al. 2008). There are morphological differences between MSC colonies and within initial colonies (Vacanti et al. 2005, Horn et al. 2008, 2010). Colonies of larger or smaller cells with prevailing round or elongated cellular morphology and with tight or disperse growth pattern can be recognized. Faster proliferating cells will overgrow slowly dividing cells. Nevertheless, variable kinetics of mitotic cell divisions between the established MSC subpopulations (i.e., their diverse migration rapidity and proliferative activity) is intensified by different methods of cell isolation/culture, different types of cell expansion media and their supplementations (i.e., various qualitative and quantitative composition/differential formulation of the media). Generally, certain media will favour different subpopulations of MSCs (Schallmoser et al. 2008, Baer et al. 2009, Baer and Geiger 2010).

### MSCs cell surface markers

Despite the variation in phenotypes of mesenchymal subpopulations, it is widely accepted that the cultured cells, regardless of the methods employed in their isolation and culture lack expression of hematopoietic antigens including clusters of differentiation (CDs): CD45 (receptor-type tyrosine-protein phosphatase C), CD34 (single-pass transmembrane sialomucin glycoprotein), CD11b (antigen-like family member B), CD14 (leukocyte-specific pathogen recognition/primitive pattern recognition co-receptor) and CD133 (prominin) expression. Simultaneously, MSCs have been reported to express: CD105 (endoglin), CD73 (5'-ribonucleotide phosphohydrolase/ecto-5'-nucleotidase; NT5E), CD90 (also known as thymus cell antigen-1/thymocyte differentiation antigen-1; Thy-1), CD29 (integrin  $\alpha_1$ ), CD44 (hyaluronate receptor), CD71 (also known as transferrin receptor), CD106 (vascular cell adhesion protein 1/vascular cell adhesion molecule 1; VCAM-1), CD166 (also called activated leukocyte cell adhesion molecule; ALCAM), STRO-1 (stromal precursor cell marker), GD2 (disialoganglioside) and CD146 (melanoma cell adhesion molecule; MCAM or cell surface glycoprotein; MUC18) (Pittenger et al. 1999, Martinez et al. 2007, Sacchetti et al. 2007). Methodology employed in the isolation of mesenchymal stem cells is heavily reliant on the ability of these cells to adhere to and subsequently proliferate on tissue culture plastic. No single, unique marker allowing for MSCs isolation has been

reported, but rather a range of composite cell surface phenotypes are utilised.

### Promotion of *in vitro* cell viability by MSC-mediated paracrine activities

An extensive study by Crisan et al. (2008) has established the links between MSCs and pericytes. Pericytes are thought to stabilize blood vessels, contribute to tissue homeostasis under physiological conditions and play an active role in response to focal tissue injury through release of bioactive molecules which have trophic and immunomodulatory properties. Based on this assumption it can be hypothesized that MSCs can exert the positive effect on the developmental capabilities and quality of *in vitro* cultured embryos. Also, Potapova et al. (2007) supported hypothesis that human MSCs (hMSCs) serve as trophic mediators for endothelial cells. They cultured hMSCs as a monolayer or as three-dimensional aggregates in hanging drops (hMSC spheroids). They showed that medium conditioned by hMSC spheroids was more effective in stimulation of umbilical vein endothelial cell proliferation, migration, and basement membrane invasion than medium conditioned by a monolayer of hMSCs. This medium also promoted *in vitro* survival rate of endothelial cells. However, it was already proved that maintaining MSCs as spheroids reduces the volume of medium required for cell culture and affects concentrations of cytokines in conditioned medium (Potapova et al. 2007).

Certainly, to be sure that the cells used for experimental embryology involving *in vitro* embryo culture (IVC) or somatic cell cloning procedures are really homogenous populations of mesenchymal stem cells, their characteristics must be performed. As mentioned previously, the broad variety of tissue sources from which MSCs are isolated, in conjunction with disparate culture conditions, has led to a lack of consensus regarding the phenotype of MSCs. In addition, differences in the formulations of media used to culture the cells, the plating density and the oxygen tension may affect the phenotype of the cell population of mesenchymal origin/lineage (Pittenger et al. 1999, Reyes et al. 2001, D'Ippolito et al. 2004).

### MSCs in tissue engineering and regenerative medicine

In recent years there has been increased interest in potential utility of MSCs in both tissue engineering and regenerative medicine that offer the novel clinical

approaches for replacement or repair of damaged, deteriorated, defective or senescent/aging cells, tissues and organs; ii) to restore, maintain or improve the tissue or organ function iii) to avoid/prevent or reverse the tissue and organ failure or insufficiency. Animal studies have provided a useful tool for defining a number of diverse potential applications for MSCs, e.g., to contribute to the functional repair of a skeletal defect (Bruder et al. 1998).

Mesenchymal cells also exhibit immunomodulatory and anti-proliferative effects on T cells, an attractive feature for cell therapy. Cultured MSCs do not express antigens of the class II major histocompatibility complex (MHC-II) on their cell surface and they suppress a primary mixed lymphocyte reaction, which has prompted the question as to whether these cells can suppress ongoing immune cell-induced reactions in humans and hence be used for the treatment of graft-versus-host disease (GVHD) (Tse et al. 2003). Therefore these cells can also be used in the preclinical and clinical research programs aimed at xenotransplantation of tissues and organs (xenograft transplantation surgery).

Several studies have provided evidence of a strong correlation between age and the proliferative potential exhibited by MSCs *in vitro*, with decreasing progenitor proliferation associated with increasing age (Clarke and McCann 1989). This diminishment could be caused by senescence of the culture-expanded cells or by terminal differentiation through the course of cell culture and passaging (Banfi et al. 2000, Vacanti et al. 2005), which may be related to epigenetic changes of mesenchymal stem cells during prolonged culture (Shibata et al. 2007). A decline in MSC function may therefore be important in several disease states (Minguell and Martinez 1983, Scopes et al. 2001) as well as in works using MSCs for *in vitro* embryo production (IVP) and somatic cell cloning.

Cultured mesenchymal cells have been shown to exhibit some unique properties that challenge the dogma that stem cells derived from the adult tissue produce only the cell lineages characteristic of tissues in which they reside. The apparent ability of MSCs to give rise to cells of multiple germ layers, however, must be examined cautiously, as undifferentiated mesenchymal cells have been shown to spontaneously express neural markers (Deng et al. 2006) as well as markers of smooth muscle cells. The mechanism by which MSCs repair damaged tissues *in vivo* is still poorly understood. However, recent evidence suggests that repair is likely achieved through paracrine factors released by mesenchymal cells rather than transdifferentiation of mesenchymal cells into specific tissue cell types (Hofstetter et al. 2002). Studies examining the efficacy of transplanted mesenchymal cells in ani-

mal models of myocardial infarction, lung injury, kidney damage and neurological diseases are ongoing, and may provide further insights into mechanisms underlying MSC-mediated tissue repair (Moscoso et al. 2005). Moreover, for these therapeutic applications, MSCs are used in an allogeneic setting that might facilitate efficient elimination of oncogenically-transformed cells by the immune system (Wagner et al. 2010a,b). Autologous application might be more prone to tumour formation. Therefore, the use of early cell passages and additional quality control, should be implemented. The danger of malignant transformation needs to be taken into account although tumour formation has not yet been described in clinical trials.

### The use of MSCs in mammals SCNT – epigenetic aspect

*In vitro* cultured fibroblast cells, which had been derived from the tissue of foetuses and adult specimens, or derived from the follicle (granulosa cells/cumulus cells) are the commonly used source of nuclear donor cells in the somatic cell cloning of mammals (Skrzyszowska et al., 2008, Jeon et al. 2008, Van Thuan et al. 2009, Zhao et al. 2010, Dai et al. 2010, Samiec and Skrzyszowska 2010a, 2012a). The degree of molecular and epigenetic differentiation of these cells, which is related both to the advanced methylation profile of DNA cytosine residues and to the lysine deacetylation profile of histones forming nucleosomal core of nuclear chromatin, often seems to make impossible converting the covalent modifications back to a totipotent state of embryonic (zygotic) cells. This leads mainly to a decrease in the abilities of differentiated fibroblast or cumulus cells for supporting the *in vitro* development of cloned embryos to the blastocyst stage (Iager et al. 2008, Costa-Borges et al. 2010, Das et al. 2010, Lee et al. 2010, Samiec and Skrzyszowska 2010b, Kim et al. 2011, Samiec et al. 2012). The MSCs exhibit the low activity of histone deacetylases (HDACs) and DNA (cytosine-5)-methyltransferases (DNMTs), which gives rise to the hyperacetylation of histone lysine moieties and to wide-range demethylation of DNA cytosine residues (Vacanti et al. 2005, Inoue et al. 2007, Martinez-Diaz et al. 2010). The last two processes impact the inhibition of transcriptional suppression of many regions in the nuclear genome of multipotent MSCs. All these properties are responsible for the high susceptibility of the MSC nuclei to correct and complete epigenetic reprogramming in the cytoplasm of NT embryo blastomeres. That is why the genome of undifferentiated stem cells may be more easily reprogrammed to

resemble the genome of the zygote, which may make stem cells more efficient as nuclear donors in the somatic cell cloning (Yamanaka et al. 2009, Dai et al. 2010).

Such possibility has been indirectly confirmed by the expression profiles of different genes at different stages of MSC/NT embryos preimplantation development which more closely mimic *in vivo*-produced pig embryos when compared with NT embryos reconstituted with fibroblast cells (Kumar et al. 2007). The panel of genes analysed involved: genes engaged in transcription and pluripotency (POU domain transcription factor *Oct4*, signal transducer and activator of transcription *Stat3*, homeobox containing transcription factor *Nanog*), genes involved in DNA methylation and histone deacetylation (DNA methyltransferase 1 *Dnmt1*, DNA methyltransferase 3a *Dnmt3a*, histone deacetylase 2 *Hdac2*) genes involved in growth factor signalling and genomic imprinting (insulin-like growth factor 2 *Igf2*, insulin-like growth factor 2 receptor *Igf2r*) as well as pro- and anti-apoptotic genes (B-cell leukemia/lymphoma 2 protein *Bcl-2* and Bcl-2-associated X protein *Bax*).

### SCNT efficiency with the use of somatic cells vs. undifferentiated and/or differentiated MSCs

Generation of cloned embryos that had originated from bovine and porcine enucleated oocytes receiving the cell nuclei of undifferentiated adult bone marrow-derived MSCs gave rise to consistently higher preimplantation development compared to creation of NT embryos using adult fibroblast cells (Kato et al. 2004, Colleoni et al. 2005).

Furthermore, porcine bone marrow MSCs were able to undergo transient and stable genetic modifications with non-viral and viral DNA vectors and were found to be an attractive nuclear donor cell type for production of transgenic cloned embryos/offspring (Colleoni et al. 2005, Bosch et al. 2006). Generally, NT blastocysts yields originated from porcine MSCs were significantly higher than NT blastocysts yields originated from adult and foetal fibroblast cells (Faast et al. 2006, Jin et al. 2007, Kumar et al. 2007, Lee et al. 2010). Interestingly and contrary to the abovementioned conclusion, Bosch et al. (2006) observed no difference in the rate of development of cloned pig embryos to the blastocyst stage when as donor nuclei served transfected fibroblast cell and genetically-modified MSC. In another study convincing of superior MSCs utility over somatic cells for cloning purposes, NT blastocysts yields originated from porcine MSCs were significantly higher than NT

blastocysts yields originated from MSCs differentiated *in vitro* into osteocytes, adipocytes and chondrocytes. Still, the developmental outcome of the latter was 2 times higher in comparison to NT embryos derived from fetal fibroblasts (Lee et al. 2010). Therefore, regarding cloning it can be stated that MSCs and MSCs subjected to differentiation are more efficient and more useful than somatic cells.

As MSCs are relatively difficult to isolate from the bone marrow of live animals, attempts were undertaken to determine whether MSCs could be isolated from the peripheral blood circulation system and subsequently used for efficient production of NT embryos. Although these cells exhibited the same antigen profile as bone marrow MSCs the enhancement in preimplantation developmental capacity of NT embryos reconstructed with blood-derived MSCs did not occur (Faast et al. 2006).

On the basis of the above-mentioned findings, it is hypothesized that the use of undifferentiated MSCs isolated from the adult bone marrow, allows to improve the preimplantation developmental potential of mammalian cloned embryos.

### Conclusions

The origin and type of nuclear donor somatic cells could be considered the prime causes affecting the developmental capability of cloned embryos, and the progressive diminishment in SCNT efficiency has been related to the molecular and epigenetic differentiation status of the nuclear donor cells (Inoue et al. 2007, Kumar et al. 2007). For this reason, the MSCs bearing a relatively undifferentiated genome might serve as suitable source of nuclear donors the epigenetic intracellular memory of which could be more faithfully reprogrammed to establish *de novo* and recapitulate the early embryonic gene expression patterns (i.e., both DNA transcriptional and mRNA translational activities) following somatic cell cloning.

It will be important to further characterize the biology of different subpopulations of MSCs and to better determine their potential in various applications including assisted reproductive technologies, experimental embryology and embryo genomic engineering. We are still not able to fully predict or control the MSC behaviour, homing potential, molecular mechanisms underlying the MSC genomic/epigenomic plasticity, multipotency level determined by adult or foetal stages of ontogenesis, proliferative activity, robust clonal self-renewal and lineage commitment, and their ability to spontaneously transform. By standardized culture conditions and the use of high-quality MSCs, their spontaneous transformation (i.e., multi-

lineage transdifferentiation, oncogenic transformation and tumour-supporting potential) may be limited or avoided *in vitro*. Due to extensive characterization of multipotent MSCs, the specific protein factors that are secreted by the MSC clonal lines may be identified, which can play a pivotal role for both developmental competencies and epigenetic DNA reprogrammability of *in vitro* fertilization-derived and somatic cell cloned embryos.

### Acknowledgements

The project was funded by the Polish National Science Centre resources allocated on the basis of decision number DEC-2011/03/D/NZ9/05537.

### References

- Baer PC, Bereiter-Hahn J, Missler C, Brzoska M, Schubert R, Gauer S, Geiger H (2009) Conditioned medium from renal tubular epithelial cells initiates differentiation of human mesenchymal stem cells. *Cell Prolif* 42: 29-37.
- Baer PC, Geiger H (2010) Mesenchymal stem cell interactions with growth factors on kidney repair. *Curr Opin Nephrol Hypertens* 19: 1-6.
- Banfi A, Muraglia A, Dozin B, Mastrogiacomo M, Cancedda R, Quarto R (2000) Proliferation kinetics and differentiation potential of ex vivo expanded human bone marrow stromal cells: Implications for their use in cell therapy. *Exp Hematol* 28: 707-715.
- Bosch P, Pratt SL, Stice SL (2006) Isolation, characterization, gene modification, and nuclear reprogramming of porcine mesenchymal stem cells. *Biol Reprod* 74: 46-57.
- Bruder SP, Kurth AA, Shea M, Hayes WC, Jaiswal N, Kadiyala S (1998) Bone regeneration by implantation of purified, culture-expanded human mesenchymal stem cells. *J Orthop Res* 16: 155-162.
- Caplan AI (2007) Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. *J Cell Physiol* 213: 341-347.
- Clarke E, McCann SR (1989) Age dependent *in vitro* stromal growth. *Bone Marrow Transplant* 4: 596-597.
- Colleoni S, Donofrio G, Lagutina I, Duchi R, Galli C, Lazzari G (2005) Establishment, differentiation, electroporation, viral transduction, and nuclear transfer of bovine and porcine mesenchymal stem cells. *Cloning Stem Cells* 7: 154-166.
- Costa-Borges N, Santaló J, Ibhnez E (2010) Comparison between the effects of valproic acid and trichostatin A on the *in vitro* development, blastocyst quality, and full-term development of mouse somatic cell nuclear transfer embryos. *Cell Reprogram* 12: 437-446.
- Crisan M, Yap S, Casteilla L, Chen CW, Corselli M, Park TS, Andriolo G, Sun B, Zheng B, Zhang L, Norotte C, Teng PN, Traas J, Schugar R, Deasy BM, Badylak S, Buhning HJ, Giacobino JP, Lazzari L, Huard J, Peault B (2008) A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell* 3: 301-313.
- Dai X, Hao J, Hou XJ, Hai T, Fan Y, Yu Y, Jouneau A, Wang L, Zhou Q (2010) Somatic nucleus reprogramming is significantly improved by *m*-carboxycinnamic acid bishydroxamide, a histone deacetylase inhibitor. *J Biol Chem* 285: 31002-31010.
- Das ZC, Gupta MK, Uhm SJ, Lee HT (2010) Increasing histone acetylation of cloned embryos, but not donor cells, by sodium butyrate improves their *in vitro* development in pigs. *Cell Reprogram* 12: 95-104.
- Deng J, Petersen BE, Steindler DA, Jorgensen ML, Laywell ED (2006) Mesenchymal stem cells spontaneously express neural proteins in culture and are neurogenic after transplantation. *Stem Cells* 24: 1054-1064.
- D'Ippolito G, Diabira S, Howard GA, Menei P, Roos BA, Schiller PC (2004) Marrow-isolated adult multilineage inducible (MIAMI) cells, a unique population of postnatal young and old human cells with extensive expansion and differentiation potential. *J Cell Sci* 117: 2971-2981.
- Faast R, Harrison SJ, Beebe LF, McIlpatrick SM, Ashman RJ, Nottle MB (2006) Use of adult mesenchymal stem cells isolated from bone marrow and blood for somatic cell nuclear transfer in pigs. *Cloning Stem Cells* 8: 166-173.
- Ho AD, Wagner W, Franke W (2008) Heterogeneity of mesenchymal stromal cell preparations. *Cytotherapy* 10: 320-330.
- Hofstetter CP, Schwarz EJ, Hess D, Widenfalk J, El Manira A, Prockop DJ, Olson L (2002) Marrow stromal cells form guiding strands in the injured spinal cord and promote recovery. *Proc Natl Acad Sci USA* 99: 2199-2204.
- Horn P, Bork S, Diehlmann A, Walenda T, Eckstein V, Ho AD, Wagner W (2008) Isolation of human mesenchymal stromal cells is more efficient by red blood cell lysis. *Cytotherapy* 10: 676-685.
- Horn P, Bokermann G, Cholewa D, Bork S, Walenda T, Koch C, Drescher W, Hutschenreuther G, Zenke M, Ho AD, Wagner W (2010) Impact of individual platelet lysates on isolation and growth of human mesenchymal stromal cells. *Cytotherapy* 12: 888-898.
- Horwitz EM, Le Blanc K, Dominici M, Mueller I, Slaper-Cortenbach I, Marini FC, Deans RJ, Krause DS, Keating A (2005) Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. *Cytotherapy* 7: 393-395.
- Iager AE, Ragina NP, Ross PJ, Beyhan Z, Cunniff K, Rodriguez RM, Cibelli JB (2008) Trichostatin A improves histone acetylation in bovine somatic cell nuclear transfer early embryos. *Cloning Stem Cells* 10: 371-379.
- Inoue K, Noda S, Ogonuki N, Miki H, Inoue S, Katayama K, Mekada K, Miyoshi H, Ogura A (2007) Differential developmental ability of embryos cloned from tissue-specific stem cells. *Stem Cells* 25: 1279-1285.
- Jeon BG, Coppola G, Perrault SD, Rho GJ, Betts DH, King WA (2008) *S*-adenosylhomocysteine treatment of adult female fibroblasts alters X-chromosome inactivation and improves *in vitro* embryo development after somatic cell nuclear transfer. *Reproduction* 135: 815-828.
- Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC, Largaespada DA, Verfaillie CM (2002) Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* 418: 41-49.
- Jin HF, Kumar BM, Kim JG, Song HJ, Jeong YJ, Cho SK, Balasubramanian S, Choe SY, Rho GJ (2007) Enhanced

- development of porcine embryos cloned from bone marrow mesenchymal stem cells. *Int J Dev Biol* 51: 85-90.
- Jones E, McGonagle D (2008) Human bone marrow mesenchymal stem cells *in vivo*. *Rheumatology (Oxford)* 47: 126-131.
- Kato Y, Imabayashi H, Mori T, Tani T, Taniguchi M, Higashi M, Matsumoto M, Umezawa A, Tsunoda Y (2004) Nuclear transfer of adult bone marrow mesenchymal stem cells: developmental totipotency of tissue-specific stem cells from an adult mammal. *Biol Reprod* 70: 415-418.
- Kim YJ, Ahn KS, Kim M, Shim H (2011) Comparison of potency between histone deacetylase inhibitors trichostatin A and valproic acid on enhancing *in vitro* development of porcine somatic cell nuclear transfer embryos. *In Vitro Cell Dev Biol Anim* 47: 283-289.
- Kumar BM, Jin HF, Kim JG, Ock SA, Hong Y, Balasubramanian S, Choe SY, Rho GJ (2007) Differential gene expression patterns in porcine nuclear transfer embryos reconstructed with fetal fibroblasts and mesenchymal stem cells. *Dev Dyn* 236: 435-446.
- Lee HS, Yu XF, Bang JI, Cho SJ, Deb GK, Kim BW, Kong IK (2010) Enhanced histone acetylation in somatic cells induced by a histone deacetylase inhibitor improved inter-generic cloned leopard cat blastocysts. *Theriogenology* 74: 1439-1449.
- Lee SL, Kang EJ, Maeng GH, Kim MJ, Park JK, Kim TS, Hyun SH, Lee ES, Rho GJ (2010) Developmental ability of miniature pig embryos cloned with mesenchymal stem cells. *J Reprod Dev* 56: 256-262.
- Martinez C, Hofmann TJ, Marino R, Dominici M, Horwitz EM (2007) Human bone marrow mesenchymal stromal cells express the neural ganglioside GD2: a novel surface marker for the identification of MSCs. *Blood* 109: 4245-4248.
- Martinez-Diaz MA, Che L, Albornoz M, Seneda MM, Collis D, Coutinho AR, El-Beirouthi N, Laurin D, Zhao X, Bordignon V (2010) Pre- and postimplantation development of swine-cloned embryos derived from fibroblasts and bone marrow cells after inhibition of histone deacetylases. *Cell Reprogram* 12: 85-94.
- Minguell JJ, Martínez J (1983) Growth pattern and function of bone marrow fibroblasts from normal and acute lymphoblastic leukemia patients. *Exp Hematol* 11: 522-526.
- Moscoso I, Centeno A, López E, Rodríguez-Barbosa JL, Santamarina I, Filgueira P, Shnchez MJ, Domínguez-Perles R, Peñuelas-Rivas G, Domenech N (2005) Differentiation "in vitro" of primary and immortalized porcine mesenchymal stem cells into cardiomyocytes for cell transplantation. *Transplant Proc* 37: 481-482.
- Oh SH, Witek RP, Bae SH, Zheng D, Jung Y, Piscaglia AC, Petersen BE (2007) Bone marrow-derived hepatic oval cells differentiate into hepatocytes in 2-acetylaminofluorene/partial hepatectomy-induced liver regeneration. *Gastroenterology* 132: 1077-1087.
- Oh SH, Witek RP, Bae SH, Darwiche H, Jung Y, Pi L, Brown A, Petersen BE (2009) Detection of transketolase in bone marrow-derived insulin-producing cells: benfotiamine enhances insulin synthesis and glucose metabolism. *Stem Cells Dev* 18: 37-46.
- Phinney DG (2007) Biochemical heterogeneity of mesenchymal stem cell populations: clues to their therapeutic efficacy. *Cell Cycle* 6: 2884-2889.
- Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR (1999) Multilineage potential of adult human mesenchymal stem cells. *Science* 284: 143-147.
- Potapova IA, Gaudette GR, Brink PR, Robinson RB, Rosen MR, Cohen IS, Doronin SV (2007) Mesenchymal stem cells support migration, extracellular matrix invasion, proliferation, and survival of endothelial cells *in vitro*. *Stem Cells* 25: 1761-1768.
- Ratajczak MZ, Kucia M, Majka M, Reza R, Ratajczak J (2004) Heterogeneous populations of bone marrow stem cells – are we spotting on the same cells from the different angles? *Folia Histochem Cytobiol* 42: 139-146.
- Ratajczak MZ, Zuba-Surma EK, Wysoczynski M, Ratajczak J, Kucia M (2008) Very small embryonic-like stem cells: characterization, developmental origin, and biological significance. *Exp Hematol* 36: 742-751.
- Reyes M, Lund T, Lenvik T, Aguiar D, Koodie L, Verfaillie CM (2001) Purification and *ex vivo* expansion of postnatal human marrow mesodermal progenitor cells. *Blood* 98: 2615-2625.
- Sacchetti B, Funari A, Michienzi S, Di Cesare S, Piersanti S, Saggio I, Tagliafico E, Ferrari S, Robey PG, Riminucci M, Bianco P (2007) Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. *Cell* 131: 324-336.
- Samiec M, Skrzyszowska M (2010a) Preimplantation developmental capability of cloned pig embryos derived from different types of nuclear donor somatic cells. *Ann Anim Sci* 10: 385-398.
- Samiec M, Skrzyszowska M (2010b) The use of different methods of oocyte activation for generation of porcine fibroblast cell nuclear-transferred embryos. *Ann Anim Sci* 10: 399-411.
- Samiec M, Skrzyszowska M (2012) Roscovitine is a novel agent that can be used for the activation of porcine oocytes reconstructed with adult cutaneous and fetal fibroblast cell nuclei. *Theriogenology* 78: 1855-1867.
- Schallmoser K, Rohde E, Reinisch A, Bartmann C, Thaler D, Drexler C, Obenauf AC, Lanzer G, Linkesch W, Strunk D (2008) Rapid large-scale expansion of functional mesenchymal stem cells from unmanipulated bone marrow without animal serum. *Tissue Eng Part C Methods* 14: 185-196.
- Scopes J, Ismail M, Marks KJ, Rutherford TR, Draycott GS, Pocock C, Gordon-Smith EC, Gibson FM (2001) Correction of stromal cell defect after bone marrow transplantation in aplastic anaemia. *Br J Haematol* 115: 642-652.
- Shibata KR, Aoyama T, Shima Y, Fukiage K, Otsuka S, Furu M, Kohno Y, Ito K, Fujibayashi S, Neo M, Nakayama T, Nakamura T, Toguchida J (2007) Expression of the p16INK4A gene is associated closely with senescence of human mesenchymal stem cells and is potentially silenced by DNA methylation during *in vitro* expansion. *Stem Cells* 25: 2371-2382.
- Skrzyszowska M, Samiec M, Słomski R, Lipiński D, Mały E (2008) Development of porcine transgenic nuclear-transferred embryos derived from fibroblast cells transfected by the novel technique of nucleofection or standard lipofection. *Theriogenology* 70: 248-259.
- Tondreau T, Dejenefte M, Meuleman N, Stamatopoulos B, Delforge A, Martiat P, Bron D, Lagneaux L (2008) Gene expression pattern of functional neuronal cells derived

- from human bone marrow mesenchymal stromal cells. *BMC Genomics* 9: 166.
- Tse WT, Pendleton JD, Beyer WM, Egalka MC, Guinan EC (2003) Suppression of allogeneic T-cell proliferation by human marrow stromal cells: implications in transplantation. *Transplantation* 75: 389-397.
- Vacanti V, Kong E, Suzuki G, Sato K, Cauty JM, Lee T (2005) Phenotypic changes of adult porcine mesenchymal stem cells induced by prolonged passaging in culture. *J Cell Physiol* 205: 194-201.
- Van Thuan N, Bui HT, Kim JH, Hikichi T, Wakayama S, Kishigami S, Mizutani E, Wakayama T (2009) The histone deacetylase inhibitor scriptaid enhances nascent mRNA production and rescues full-term development in cloned inbred mice. *Reproduction* 138: 309-317.
- Wagner W, Ho AD (2007) Mesenchymal stem cell preparations – comparing apples and oranges. *Stem Cell Rev* 3: 239-248.
- Wagner W, Bork S, Lepperdinger G, Jousen S, Ma N, Strunk D, Koch C (2010a) How to track cellular aging of mesenchymal stromal cells? *Aging (Albany NY)* 2: 224-230.
- Wagner W, Ho AD, Zenke M (2010b) Different facets of aging in human mesenchymal stem cells. *Tissue Eng Part B Rev* 16: 445-453.
- Yamanaka K, Sugimura S, Wakai T, Kawahara M, Sato E (2009) Acetylation level of histone H3 in early embryonic stages affects subsequent development of miniature pig somatic cell nuclear transfer embryos. *J Reprod Dev* 55: 638-644.
- Zhao J, Hao Y, Ross JW, Spate LD, Walters EM, Samuel MS, Rieke A, Murphy CN, Prather RS (2010) Histone deacetylase inhibitors improve *in vitro* and *in vivo* developmental competence of somatic cell nuclear transfer porcine embryos. *Cell Reprogram* 12: 75-83.