Title:

Bone marrow as a source of stem cells and germ cells? Perspectives for transplantation.

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Abstract:

Recent publications have suggested the existence of germ stem cells in the mouse at postnatal stages. The mechanism of *de novo* oocyte formation is proposed to involve a contribution from the bone marrow to the germ cell pool, via the bloodstream. Critical examination of the data underpinning these contentious claims is under way from a reproductive biology perspective, but little has been said about the nature this elusive bone marrow population with germ cell potential. Furthermore, whilst the prospect of marrow-derived germ cells may appear propitious for fertility applications, its wider impact on transplantation medicine remains to be considered. This paper examines the evidence leading to the current debate, and considers the potential implications of such findings for the field of bone marrow transplantation.

Keywords: Bone marrow, stem cell, transplantation, germ cell, ethics.

Extragonadal germ stem cells in the adult ?

The hypothesis of adult germ stem cells

A debate was launched following the publication of two studies by Johnson and colleagues in the past two years (Johnson et al. 2004), (Johnson et al. 2005). The earlier paper (Johnson et al. 2004) reported observations questioning the long-established dogma that the number of mammalian oocytes is fixed by the start of postnatal life. Contrary to male germ cells, the production of female germ cells is generally considered to be limited and the initial oocyte reserve gets gradually depleted over the phase of reproductive competence, the menopause marking the exhaustion of this finite pool (Gosden et al. 1983), (McClellan et al. 2003). However, the paper by Johnson and colleagues describes signs of preservation and proliferation of germline stem cells in postnatal mouse ovaries, thus opening the possibility of a postnatal contribution to the oocyte reserve. Another report using a similar mouse model recently confirmed that in ovaries from 7- to 100-day old mice, the mean numbers of primordial follicles were not significantly depleted, supporting some degree of follicle renewal in postnatal and adult mouse ovaries (Kerr et al. 2006). Transplantation experiments suggesting colonisation of grafted ovarian tissue by labelled oocytes from GFP-expressing hosts led the authors to propose that immature germ cells present in the adult may be able to generate *de novo* oocytes in a favourable environment. These elusive germ cell progenitors in the ovary were reported to express the stage specific embryonic antigen-1 (SSEA1), and germ cell markers Oct4, Mvh, Dazl and Stella, albeit at a low level (Johnson et al. 2005).

Bone marrow contribution to germ cell pool?

In a subsequent paper, Johnson et al. described their attempt to identify the postulated germ cell progenitor (referred to as germ stem cell (GSC)) (Johnson et al. 2005). Based on the hypothesis of an extra-ovarian source of precursors able to home to the ovaries and contribute new oocytes, the authors analysed samples of bone marrow and peripheral blood by RT-PCR. They detected the expression of germline markers in these tissues, and controversially suggested that GSCs may be provided by adult bone marrow through the circulation. Further corroboration was gained *in vivo* when animals depleted of their

endogenous oocytes before bone marrow transplantation showed signs of new germ cells developing from a donor origin. However, interpretation of these results necessitates some caution in the light of past transplantation experiments, which have showed that donor bone marrow stem cells can undergo spontaneous fusion with differentiated host cells (Weimann et al. 2003), (Alvarez-Dolado et al. 2003).

A recent report (Eggan et al. 2006) somewhat contradicts the claim that precursors in peripheral blood may generate new functional germ cells. Using a model of parabiosis allowing blood circulation between a GFP-labelled and a non-labelled mouse, this study showed no obvious cross-contribution from the mouse partner's blood flow to the pool of available oocytes, with the exception of infiltrated cd45-positive cells which did not exhibit germline features. As called for by many commentators (Byskov et al. 2005), (Bukovsky 2005), (Telfer et al. 2005), (Gosden 2004), more data is needed to resolve this question, in particular the proof of *bona fide* differentiation from these germ stem cells through the generation of functional oocytes with reproductive potential.

Bone marrow: a germ stem cell reservoir?

Although the debate is intense around the experimental caveats and extrapolations presented in these initial studies, little has been said about the cells proposed as the extra-gonadal source of germ stem cells. The nature of the bone marrow subpopulation identified by Johnson et al. is quite intriguing as, contrary to what may have been expected, it does not correspond to classical stem cell populations.

Hematopoietic stem cells

Existing studies have drawn attention to a possible plasticity between the hematopoietic stem cell (HSC) and the germ cell lineage. Hematopoietic progenitors and primordial germ cells develop in close proximity during embryogenesis, and *in vitro* experiments have demonstrated that primordial germ cells can exhibit hematopoietic potential and form erythroid derivatives (Rich 1995). It is unclear whether the HSC is the cell type involved in the

phenomenon observed by Johnson et al., as the elusive germ stem cells appear in the lin-/Sca1-/c-kit+ fraction. The lin- (or lineage marker-negative) fraction represents bone marrow depleted of cells expressing markers of mature hematopoietic lineages (Okada et al. 1992), and is therefore enriched for uncommitted progenitors (Spangrude et al. 1988). Furthermore, it has been established that primitive HSCs express c-kit (cd117) (Okada et al. 1991), a feature shared with germ cells (Manova et al. 1990), (Hutt et al. 2006). However, unlike the population reported to contain GSCs (Johnson et al. 2005), primitive HSCs also express Sca1 and are therefore lin-/Sca1+/c-kit+ (fig.1), whereas Sca1 is absent in more committed progenitor populations (Okada et al. 1992), (Zhang et al. 1995), (Morita et al. 2006), (McKinstry et al. 1997). For instance, a sorting strategy based on lin-/Sca1-/c-kit+ (similar to that described by Johnson et al.) has been used to enrich for myeloid precursors of the macrophage/osteoclast lineage (Muguruma and Lee 1998).

Hematopoietic stem cells have also been isolated within the side population ('SP') of cells in the marrow. SP cells are defined by their ability to efflux dyes, which is considered a property common to stem cells across many tissues. SP cells can be isolated from the bone marrow and the testis, where they allow significant enrichment for HSCs and germ cell progenitors, respectively (Goodell et al. 1996), (Lassalle et al. 2004), (Falciatori et al. 2004). However, the marrow SP population typically appears within the lin-/Sca1+/c-kit+ fraction (Pearce et al. 2004) (fig.1).

Non hematopoietic stem cells

An alternative marrow stem cell type which could be envisaged as a source of GSCs is the mesenchymal stem cell ('MSC'). In fact, the method referenced by Johnson et al. to prepare bone marrow cultures had initially been developed to isolate MSCs (Meirelles Lda and Nardi 2003), and it generated an adherent culture passaged as a monolayer (Johnson et al. 2005). MSCs are multipotent progenitors with established adipogenic, osteogenic and chondrogenic differentiation ability (Pittenger et al. 1999), and are believed to exhibit some differentiation markers for lineages beyond mesenchymal cell types (Hermann et al. 2004), (Deng et al. 2006). A recent study using adherent stromal cells from mouse bone marrow suggests that expression of markers specific for male germ cells can be induced in these cells (Nayernia et

al. 2006). The bone marrow isolated in this study was collected from a transgenic mouse model in which GFP expression is under the control of the Stra8 promoter, a retinoic acid-responsive gene expressed in the male germ cell lineage (Oulad-Abdelghani et al. 1996). *In vitro* treatment with retinoic acid led to the appearance of a small fraction of GFP-positive cells indicating activation of Stra8 expression in these cells, and the detection of germline markers such as Oct4, Stella, Dazl and Fragilis (Saitou et al. 2002). These marrow-derived cells were also reported to be able to colonise the gonad after transplantation. Although the analysis by RT-PCR doesn't allow unequivocal quantification of these changes in the gene expression profile, this work provides a first basis to further investigate the germ cell potential in these cells. The study is centred on MSCs, although in the absence of any specific cell sorting the precise nature of the stromal cells able to express Stra8 remains unclear. It seems unlikely, however, that the cells described by Johnson et al. correspond to this stromal fraction, as mouse MSCs are reported to be found within the lin-/Sca1+/c-kit- fraction (Baddoo et al. 2003), (Deng et al. 2006), (Anjos-Afonso et al. 2004) (fig.1). Also, the reported germ cell-enriched fraction does not appear to fit the description of multipotent adult progenitor cells (MAPCs) identified in the bone marrow. Although MAPCs have been reported to express SSEA-1 and low levels of Oct4, which are both also expressed in primordial germ cells (McLaren and Durcova-Hills 2001), (Saitou et al. 2002), they are weakly positive for Sca1 and negative for c-kit (Jiang et al. 2002) (fig.1).

A recent report has identified an equally unanticipated somatic source of germ cell precursors. Using a porcine model, Dyce et al. have shown that stem cells isolated from foetal skin can form oocyte-like cells *in vitro* (Dyce et al. 2006). This study illustrates the current search for extra-gonadal cells with germ stem cell potential, although the physiological significance of such findings is unclear. The possible route of delivery for skin-derived stem cells to the ovary appears enigmatic, whereas cells of marrow origin could colonise distant organs through the circulation. Others have shown that adult human female germ cells derived from the ovarian surface epithelium can enter the bloodstream (Bukovsky et al. 1995), (Bukovsky et al. 2004), and speculated that such cells may contaminate the blood and bone marrow (Bukovsky 2005), which is known to be seeded with many mobile stem cell populations (Hirschi and Goodell 2002), (Roufosse et al. 2004), (Palermo et al. 2005).

Refining the molecular profile of the GSC fraction is now necessary in order to assess its physiological relevance, and evaluate its relationship to established stem cell populations. Recently, a hypothesis has been raised that the prospect of germline regeneration, while unlikely to occur in mice, may have some basis in primates (Hutt and Albertini 2006).

Considerations for transplantation:

The hypothetical existence of adult germ stem cells, at the crossroads of developmental biology, stem cell biology and reproductive biology, is still very much open, as the experimental results and interpretation underpinning this debate remain highly contentious (Telfer et al. 2005), (Eggan et al. 2006), (Bukovsky 2005). As more investigators enter this debate, the data presented in Johnson et al.'s reports will no doubt be analysed and tested in great detail. Beyond the technical and experimental arguments, it is interesting to take a step back and envisage the impact such a challenging concept may have from a clinical perspective. Experts and observers of the field have commented on the consequences of such reports for reproductive medicine (Bukovsky 2005), (Gosden 2004), (Powell 2006), (Couzin 2004), (Kayisli and Seli 2006). Following the paradigm of regenerative medicine using adult stem cells to repair somatic tissues such as bone or cardiac muscle (Quarto et al. 2001), (Zhang et al. 2005), it is tempting to imagine that adult germ stem cells could in the future represent a potential source to treat infertility issues (Kayisli and Seli 2006). This possibility has already been formulated in the case of young cancer patients, as autologous blood or bone marrow harvested during ovarian oogenesis (potentially containing germ cells) prior to anti-cancer chemotherapy could be used post-chemotherapy (Bukovsky 2005). Such hopes are undoubtedly premature at this stage, as the observations presented by Johnson et al. and their relevance to the human are to be confirmed (Telfer et al. 2005), (Bukovsky 2005), (Byskov et al. 2005).

However, such speculations around possible therapeutic benefits to patients draw attention to a corollary issue regarding the use of bone marrow donations. If more detailed reports come

to support the existence of a germ cell progenitor in the marrow, and if it is conceivable as envisaged by Johnson et al. that this precursor is able to form new germ cells with reproductive potential, this raises the possibility that bone marrow transplant could indirectly lead to a germ cell transplant. In other words, could a bone marrow recipient have an offspring conceived from a bone marrow donor-derived germ cell? Further transplantation experiments in mouse models are now needed to establish whether cells originating from transplanted bone marrow can develop into genuine germ cells of the recipient, and demonstrate reproductive ability. The medical and societal consequences of such a biological possibility could be significant, particularly in cases where donors and recipients are related. Moreover, if the debate was to continue and enter the public arena, how would it affect bone marrow donations? Issues raised by such contentious concepts are both of scientific and ethical nature, as they could significantly affect both donors' and a recipients' perspective on transplantation. As with all challenges created by advances in stem cell research, complex ethical questions are inevitable (McLaren 2001), (Daar and Sheremeta 2003). Consequently, the evolution of this fierce debate involving stem cell biologists and reproductive biologists will also be of particular relevance to medical ethicists.

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REFERENCES

Alvarez-Dolado M, Pardal R, Garcia-Verdugo JM, Fike JR, Lee HO, Pfeffer K, Lois C, Morrison SJ and Alvarez-Buylla A (2003) Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. Nature 425: 968-73

Anjos-Afonso F, Siapati EK and Bonnet D (2004) In vivo contribution of murine mesenchymal stem cells into multiple cell-types under minimal damage conditions. J Cell Sci 117: 5655-64

Baddoo M, Hill K, Wilkinson R, Gaupp D, Hughes C, Kopen GC and Phinney DG (2003) Characterization of mesenchymal stem cells isolated from murine bone marrow by negative selection. J Cell Biochem 89: 1235-49

Bukovsky A, Keenan JA, Caudle MR, Wimalasena J, Upadhyaya NB, Van Meter SE (1995) Immunohistochemical studies of the adult human ovary: possible contribution of immune and epithelial factors to folliculogenesis. Am J Reprod Immunol 33:323-40

Bukovsky A, Caudle MR, Svetlikova M and Upadhyaya NB (2004) Origin of germ cells and formation of new primary follicles in adult human ovaries. Reprod Biol Endocrinol 2: 20

Bukovsky A (2005) Can ovarian infertility be treated with bone marrow- or ovary-derived germ cells? Reprod Biol Endocrinol 3: 36

Byskov AG, Faddy MJ, Lemmen JG and Andersen CY (2005) Eggs forever? Differentiation 73: 438-46

Couzin J (2004) Reproductive biology. Textbook rewrite? Adult mammals may produce eggs after all. Science 303: 1593

Daar AS and Sheremeta L (2003) The science of stem cells: ethical, legal and social issues. Exp Clin Transplant 1: 139-46

Deng J, Petersen BE, Steindler DA, Jorgensen ML and Laywell ED (2006) Mesenchymal stem cells spontaneously express neural proteins in culture and are neurogenic after transplantation. Stem Cells 24: 1054-64

Dyce PW, Wen L and Li J (2006) In vitro germline potential of stem cells derived from fetal porcine skin. Nat Cell Biol 8: 384-90

Eggan K, Jurga S, Gosden R, Min IM and Wagers AJ (2006) Ovulated oocytes in adult mice derive from non-circulating germ cells. Nature 441: 1109-14

Falciatori I, Borsellino G, Haliassos N, Boitani C, Corallini S, Battistini L, Bernardi G, Stefanini M and Vicini E (2004) Identification and enrichment of spermatogonial stem cells displaying side-population phenotype in immature mouse testis. Faseb J 18: 376-8

Goodell MA, Brose K, Paradis G, Conner AS and Mulligan RC (1996) Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. J Exp Med 183: 1797-806

Gosden RG (2004) Germline stem cells in the postnatal ovary: is the ovary more like a testis? Hum Reprod Update 10: 193-5

Gosden RG, Laing SC, Felicio LS, Nelson JF and Finch CE (1983) Imminent oocyte exhaustion and reduced follicular recruitment mark the transition to acyclicity in aging C57BL/6J mice. Biol Reprod 28: 255-60

Hermann A, Gastl R, Liebau S, Popa MO, Fiedler J, Boehm BO, Maisel M, Lerche H, Schwarz J, Brenner R and Storch A (2004) Efficient generation of neural stem cell-like cells from adult human bone marrow stromal cells. J Cell Sci 117: 4411-22

Hirschi KK and Goodell MA (2002) Hematopoietic, vascular and cardiac fates of bone marrow-derived stem cells. Gene Ther 9: 648-52

Hutt KJ, Albertini DF (2006) Clinical applications and limitations of current ovarian stem cell research: a review. J Exp Clin Assist Reprod 3:6

Hutt KJ, McLaughlin EA and Holland MK (2006) KIT/KIT Ligand in Mammalian Oogenesis and Folliculogenesis: Roles in Rabbit and Murine Ovarian Follicle Activation and Oocyte Growth. Biol Reprod

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 and Verfaillie CM (2002) Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 418: 41-9

Johnson J, Bagley J, Skaznik-Wikiel M, Lee HJ, Adams GB, Niikura Y, Tschudy KS, Tilly JC, Cortes ML, Forkert R, Spitzer T, Iacomini J, Scadden DT and Tilly JL (2005) Oocyte generation in adult mammalian ovaries by putative germ cells in bone marrow and peripheral blood. Cell 122: 303-15

Johnson J, Canning J, Kaneko T, Pru JK and Tilly JL (2004) Germline stem cells and follicular renewal in the postnatal mammalian ovary. Nature 428: 145-50

Kayisli UA and Seli E (2006) Stem cells and fertility: what does the future hold? Curr Opin Obstet Gynecol 18: 338-43

Kerr JB, Duckett R, Myers M, Britt KL, Mladenovska T and Findlay JK (2006) Quantification of healthy follicles in the neonatal and adult mouse ovary: evidence for maintenance of primordial follicle supply. Reproduction 132: 95-109

Lassalle B, Bastos H, Louis JP, Riou L, Testart J, Dutrillaux B, Fouchet P and Allemand I (2004) 'Side Population' cells in adult mouse testis express Bcrp1 gene and are enriched in spermatogonia and germinal stem cells. Development 131: 479-87

Manova K, Nocka K, Besmer P and Bachvarova RF (1990) Gonadal expression of c-kit encoded at the W locus of the mouse. Development 110: 1057-69

McClellan KA, Gosden R and Taketo T (2003) Continuous loss of oocytes throughout meiotic prophase in the normal mouse ovary. Dev Biol 258: 334-48

McKinstry WJ, Li CL, Rasko JE, Nicola NA, Johnson GR and Metcalf D (1997) Cytokine receptor expression on hematopoietic stem and progenitor cells. Blood 89: 65-71

McLaren A (2001) Ethical and social considerations of stem cell research. Nature 414: 129-31
McLaren A and Durcova-Hills G (2001) Germ cells and pluripotent stem cells in the mouse. Reprod Fertil Dev 13: 661-4

Meirelles Lda S and Nardi NB (2003) Murine marrow-derived mesenchymal stem cell: isolation, in vitro expansion, and characterization. Br J Haematol 123: 702-11

Morita Y, Ema H, Yamazaki S and Nakauchi H (2006) Non - side-population hematopoietic stem cells in mouse bone marrow. Blood [Epub ahead of print]

Muguruma Y and Lee MY (1998) Isolation and characterization of murine clonogenic osteoclast progenitors by cell surface phenotype analysis. Blood 91: 1272-9

Nayernia K, Lee JH, Drusenheimer N, Nolte J, Wulf G, Dressel R, Gromoll J and Engel W (2006) Derivation of male germ cells from bone marrow stem cells. Lab Invest 86: 654-63

Okada S, Nakauchi H, Nagayoshi K, Nishikawa S, Miura Y and Suda T (1992) In vivo and in vitro stem cell function of c-kit- and Sca-1-positive murine hematopoietic cells. Blood 80: 3044-50

Okada S, Nakauchi H, Nagayoshi K, Nishikawa S, Nishikawa S, Miura Y and Suda T (1991) Enrichment and characterization of murine hematopoietic stem cells that express c-kit molecule. Blood 78: 1706-12

Oulad-Abdelghani M, Bouillet P, Decimo D, Gansmuller A, Heyberger S, Dolle P, Bronner S, Lutz Y and Chambon P (1996) Characterization of a premeiotic germ cell-specific cytoplasmic protein encoded by Stra8, a novel retinoic acid-responsive gene. J Cell Biol 135: 469-77

Palermo AT, Labarge MA, Doyonnas R, Pomerantz J and Blau HM (2005) Bone marrow contribution to skeletal muscle: a physiological response to stress. Dev Biol 279: 336-44

Pearce DJ, Ridler CM, Simpson C and Bonnet D (2004) Multiparameter analysis of murine bone marrow side population cells. Blood 103: 2541-6

Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S and Marshak DR (1999) Multilineage potential of adult human mesenchymal stem cells. Science 284: 143-7

Powell K (2006) Born or made? Debate on mouse eggs reignites. Nature 441: 795

Quarto R, Mastrogiacomo M, Cancedda R, Kutepov SM, Mukhachev V, Lavroukov A, Kon E and Marcacci M (2001) Repair of large bone defects with the use of autologous bone marrow stromal cells. N Engl J Med 344: 385-6

Rich IN (1995) Primordial germ cells are capable of producing cells of the hematopoietic system in vitro. Blood 86: 463-72

Roufosse CA, Direkze NC, Otto WR and Wright NA (2004) Circulating mesenchymal stem cells. Int J Biochem Cell Biol 36: 585-97

Saitou M, Barton SC and Surani MA (2002) A molecular programme for the specification of germ cell fate in mice. Nature 418: 293-300

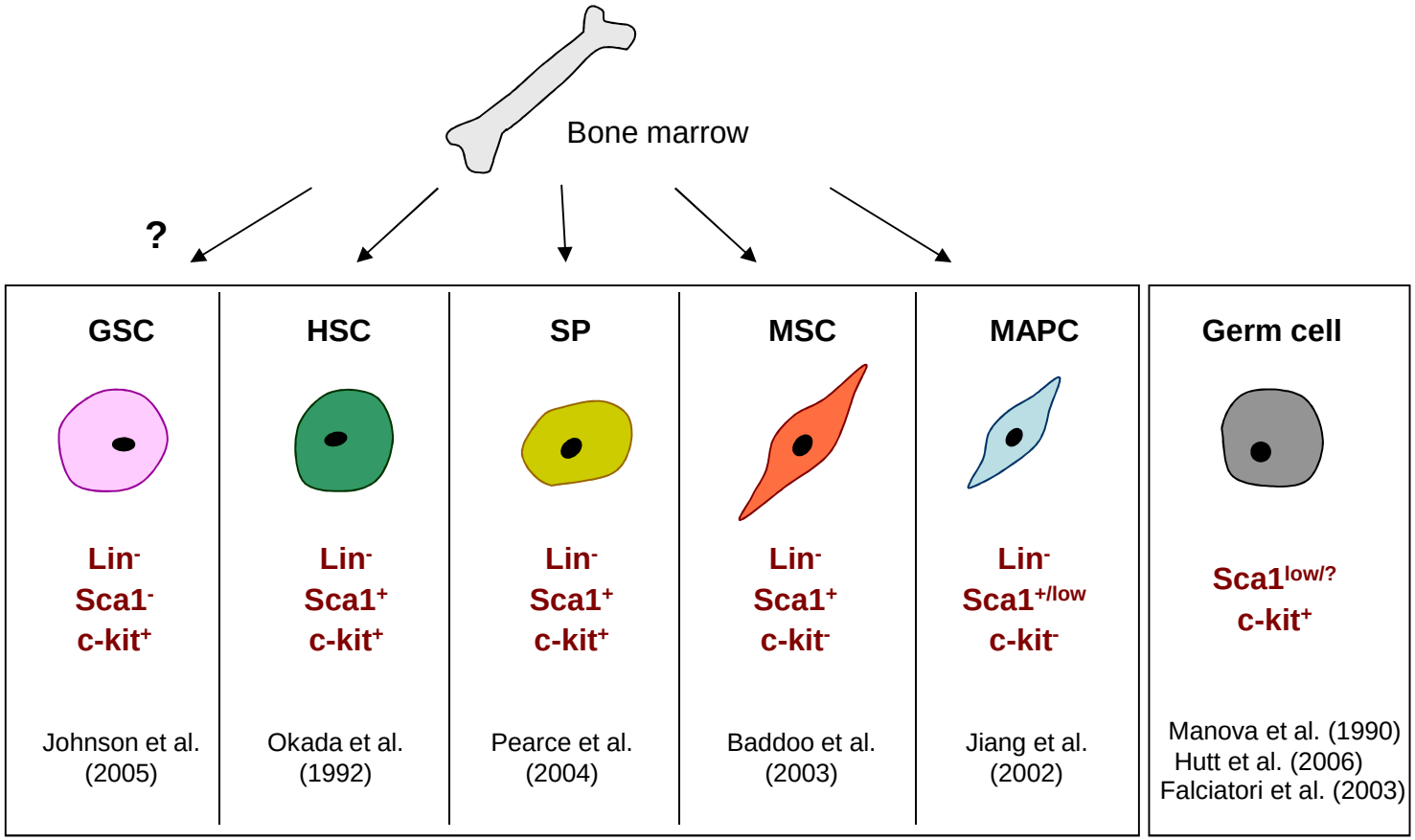
Spangrude GJ, Heimfeld S and Weissman IL (1988) Purification and characterization of mouse hematopoietic stem cells. Science 241: 58-62

Telfer EE, Gosden RG, Byskov AG, Spears N, Albertini D, Andersen CY, Anderson R, Braw-Tal R, Clarke H, Gougeon A, McLaughlin E, McLaren A, McNatty K, Schatten G, Silber S and Tsafriri A (2005) On regenerating the ovary and generating controversy. Cell 122: 821-2

Weimann JM, Johansson CB, Trejo A and Blau HM (2003) Stable reprogrammed heterokaryons form spontaneously in Purkinje neurons after bone marrow transplant. Nat Cell Biol 5: 959-66

Zhang S, Jia Z, Ge J, Gong L, Ma Y, Li T, Guo J, Chen P, Hu Q, Zhang P, Liu Y, Li Z, Ma K, Li L and Zhou C (2005) Purified human bone marrow multipotent mesenchymal stem cells regenerate infarcted myocardium in experimental rats. Cell Transplant 14: 787-98

Zhang Y, Harada A, Bluethmann H, Wang JB, Nakao S, Mukaida N and Matsushima K (1995) Tumor necrosis factor (TNF) is a physiologic regulator of hematopoietic progenitor cells: increase of early hematopoietic progenitor cells in TNF receptor p55-deficient mice in vivo and potent inhibition of progenitor cell proliferation by TNF alpha in vitro. Blood 86: 2930-7


Bone marrow
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GSC
HSC
SP
MSC
MAPC
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Baddoo et al. (2003)
Jiang et al. (2002)
Manova et al. (1990)
Hutt et al. (2006)
Falciatori et al. (2003)