Developmental Biology 477 (2021) 117–132

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

Diverse cellular origins of adult blood vascular endothelial cells

Jorge B. Aquino *, Romina Sierra, Laura A. Montaldo

CONICET-Universidad Austral, Instituto de Investigaciones en Medicina Traslacional (IIMT), Developmental Biology & Regenerative Medicine Laboratory, Argentina

ARTICLE INFO

Keywords:
Endothelial progenitor cells
Bone marrow stromal cells
CAR cells
GLAST
Plasticity
Epigenetics
Hypoxia
Exosomes
scRNA-seq
Wnt1

ABSTRACT

During embryonic stages, vascular endothelial cells (ECs) originate from the mesoderm, at specific extraembryonic and embryonic regions, through a process called vasculogenesis. In the adult, EC renewal/replacement mostly depend on local resident ECs or endothelial progenitor cells (EPCs). Nevertheless, contribution from circulating ECs/EPCs was also reported. In addition, cells lacking from EC/EPC markers with *in vitro* extended plasticity were shown to originate endothelial-like cells (ELCs). Most of these cells consist of mesenchymal stromal progenitors, which would eventually get mobilized from the bone marrow after injury. Based on that, current knowledge on different mouse and human bone marrow stromal cell (BM-SC) subpopulations, able to contribute with mesenchymal stromal/stem cells (MSCs), is herein reviewed. Such analyses underline an unexpected heterogeneity among sinusoidal $LepR^{+}$ stromal/CAR cells. For instance, in a recent report a subgroup of $LepR^{+}$ stromal/CAR progenitors, which express GLAST and is traced in $Wnt1^{Cre}$;$R26R^{Tom}$ mice, was found to contribute with ELCs *in vivo*. These GLAST $^{+}$ $Wnt1^{+}$ BM-SCs were shown to get mobilized to the peripheral blood and to contribute with liver regeneration. Other sources of ELCs, such as adipose, neural and dental pulp tissues, were also published. Finally, mechanisms likely involved in the enhanced cellular plasticity properties of bone marrow/adipose tissue stromal cells, able to originate ELCs, are assessed. In the future, strategies to analyze the *in vivo* expression profile of stromal cells, with MSC properties, in combination with screening of active genomic regions at the single cell-level, during early postnatal development and/or after injury, will likely help understanding properties of these ELC sources.

1. Introduction

Blood vascular endothelial cells (ECs) are squamous epithelial cells covering the inner surface of blood vessels (Cines et al., 1998). They most frequently constitute a continuous layer, which allows the transcellular transport of molecules. These cells can also have fenestrations or pores, more or less dynamic. EC fenestrations allow for greater molecular interchange in between the circulating blood and the underlying connective tissue. In some capillaries, the endothelial layer can be completely devoid of fenestrations, for example in the brain parenchyma, while in other tissues, such as the endocrine glands, it has very abundant fenestrations. Within the epithelium, ECs synthesize its basal lamina, a structure that in exceptional cases may present discontinuities. For example, the epithelium of hepatic capillaries (called liver sinusoids) shows many open spaces, completely devoid of cellular components and basal lamina. In addition, in the bone marrow capillaries/sinusoids some transitory pores are formed by megakaryocytes that reach the vessel lumen, or by other bone marrow cells, opening their way to the blood circulation.

Several ECs origins have been reported. Their embryonic sources will first be discussed. Next, the current knowledge about their origins in the adult will be reviewed: they are most often derived from local endothelial progenitor cells (EPCs), which present a hierarchical relationship with each other. Distant EC sources, including adult circulating ECs/EPCs (CECs) and other progenitors, play a minor role in endothelial cell turnover/endothelial layer repair *in vivo*. The bone marrow is a source of CECs and it was shown that this tissue contains stromal progenitors with properties of extended plasticity and the ability to get mobilized and originate endothelial like cells (ELCs). In this article, different mouse and human bone marrow stromal cell (BM-SC) subpopulations, capable of contributing mesenchymal stromal/stem cells (MSCs) *in vitro* are reviewed. In this regard, the evidences of an *in vivo* origin of ELCs in a subpopulation of GLAST $^{+}$ $Wnt1^{+}$ BM-SCs are evaluated. ELCs were also obtained from adipose, neural and dental pulp tissues. Finally, some mechanisms probably involved in the acquisition/maintenance of enhanced plasticity in adult stromal cells are also analyzed.

* Corresponding author. CONICET-Universidad Austral, Instituto de Investigaciones en Medicina Traslacional (IIMT), Developmental Biology & Regenerative Medicine Lab, Av. Peron 1500, B1629AHJ Derqui-Pilar, Buenos Aires, Argentina.
E-mail address: jaquino@austral.edu.ar (J.B. Aquino).

https://doi.org/10.1016/j.ydbio.2021.05.010
Received 17 December 2020; Received in revised form 26 April 2021; Accepted 14 May 2021
Available online 26 May 2021

2. Embryonic origin of ECs

Vascular and hematopoietic cells emerge and develop together within the **primitive yolk sac** in vertebrates, as seen in human, mouse, chicken, and zebrafish (Cines et al., 1998; Dyer et al., 2001; Oberlin et al., 2002; Risau and Flamme, 1995; Lenard et al., 2015; Palis et al., 1995; Gomez Perdiguero et al., 2015; McGrath et al., 2015; Ueno and Weissman, 2006; Dzierzak and Bigas, 2018). In avian embryos, some EPCs (named angioblasts) differentiate from the Ets1$^+$ fibronectin$^+$ Flk-1/VEGFR2$^+$ cells of the splanchnopleuric mesoderm and are the first vascular cells to be generated (Risau and Flamme, 1995). They form clusters, with a core made up by hematopoietic progenitors (HPCs), known as blood islands. All through ***vasculogenesis*** (*de novo* origin of vessels), angioblasts and differentiating ECs continue to express fibronectin and Flk-1. In addition, they begin to express Cdh5/VE-cadherin, Flt-1/VEGFR1 and Tie2/Tek, and share some markers with hematopoietic cells, including CD31 and Ets1. In different mouse strains and in fish, fibronectin was shown to be necessary for adequate early vascular formation (Astrof and Hynes, 2009).

In the **mouse**, vasculogenesis begins in the anterior yolk sac. In this region, diverse mesodermal cells, derived from the most posterior levels of the primitive streak, give rise to clusters of prospective endothelial and hematopoietic cells (Dyer et al., 2001; McGrath et al., 2015; Ueno and Weissman, 2006; Lawson et al., 1991). The first yolk sac blood islands/clusters are observed on embryonic day 7.5/8.0 (E7.5/8.0) (Haar and Ackerman, 1971). A more modern blood islands model for vasculogenesis suggests that a complete band of erythroid progenitors is located within the visceral yolk sac, in the vicinity of scattered endothelial cells (Ferkowicz and Yoder, 2005). In mouse, the first angioblast and endothelial marker is Flk-1 (Palis et al., 1995; Huber et al., 2004). Shortly thereafter, during vascular morphogenesis/maturation, ECs express other markers such as CD31, CD34, Flt-1 and later on Tie2 (Drake and Fleming, 2000). In birds and mouse, the initial stimulus for angioblast formation is originated in the endoderm (Risau and Flamme, 1995; Palis et al., 1995). In mice, Indian hedgehog (Ihh) is considered a relevant endoderm-derived morphogen that induces vasculogenesis, and it was shown to upregulate CD31, CD34, Flk-1 and vWF (von Willebrand factor) expression in the mesoderm of the primitive yolk sac (Dyer et al., 2001).

Soon after, first ***intraembryonic vasculogenesis*** occurs at the stage 1-somite. These angioblasts originate in the mesoderm and few of them are associated with HPCs in paraortic splachnopleura. Both types of angioblasts (in contact or not with HPCs; named as primordial and hemogenic, respectively) migrate and fuse with each other and form a primary vascular plexus, in a process called vascular anastomosis (Lenard et al., 2015). Through quail/chick *in vivo* cross-transplantation experiments, Pardanaud et al. (1996) showed two independent origins of trunk endothelial lineages: splanchnopleural mesoderm and paraxial mesoderm (somites). Splanchnopleural mesoderm gives rise to the vessels found in the floor of the aorta (where hematopoietic clusters are located in bird embryos), as well as the entire vasculature of visceral organs, through a process of vasculogenesis. On the other hand, the paraxial mesoderm originates the intersomitic arteries, the cardinal vein, and the endothelium of the neural tube, the body wall, the kidney and the roof and sides of the aorta. Thus, vessels located farther from the paraxial mesoderm originate through a process known as *angiogenesis* (sprouting from previously arose vessels). To analyze sources of ECs in the mouse, mouse/quail crossover transplantation experiments were performed. Mouse somite orthotopic grafts migrate and vascularized the neural tube, limbs and mesonephros of quail embryos (Ambler et al., 2001). In other experiments, mouse limb buds or visceral rudiments were cross-transplanted into the coelomic cavity of host quail (Pudliszewski and Pardanaud, 2005). These studies were able to demonstrate that the processes of vasculogenesis and angiogenesis in mouse are similar to those previously described in birds. In this regard, a difference found between mouse and birds is that hematopoietic clusters form in mice not only at ventral but also in the dorsal part of the aorta (Taoudi and Medvinsky, 2007).

In mouse, at E9.0 (7-to-10 somites stages), the primitive vascular network of the visceral yolk sac connects with its embryonic counterpart (Palis et al., 1995). In the adult, only a very small fraction of the original components of the **vessel plexus** is maintained: most of them regress before blood begins to circulate, due to the absence of survival signals (Cines et al., 1998; Lenard et al., 2015; Franco et al., 2015). The pruning and regression of vessels has been well analyzed in mouse (Franco et al., 2015), rat (Hughes and Chang-Ling, 2000) and zebrafish (Lenard et al., 2015) vasculature. It could thus be shown that the initial regression of the vessels depends mainly on mobilization and re-organization of endothelial cells, rather than on their apoptosis (Franco et al., 2015; Hughes and Chang-Ling, 2000). Therefore, different origins and tissue-specific morphogenetic signals could explain the heterogeneity of ECs.

After much controversy in this regard, current evidence suggest that primitive ECs and erythroid-myeloid cells do originate from different progenitors derived from the posterior primitive streak (E7.5), which differentiate in close association (McGrath et al., 2015; Ueno and Weissman, 2006; Palis et al., 1999). Therefore, there is little *in vivo* evidence for the existence of hemangioblasts, as defined by Murray in 1932 (Lacaud and Kouskoff, 2017). By this term he referred to some cells originating from primitive mesoderm capable of giving rise to all the cells of the blood islands. New evidence based on scRNA-seq studies in mice confirms a **hemogenic EC origin of the first HSCs**, during the embryonic intermediate stages (Baron et al., 2018), which confirms the previous literature obtained from birds, fish, mice and humans (Oberlin et al., 2002; Risau and Flamme, 1995; Azevedo Portilho and Pelajo-Machado, 2018; Boisset et al., 2010; de Bruijn et al., 2000; Chen et al., 2011; Medvinsky and Dzierzak, 1996; Ivanovs et al., 2011; Bertrand et al., 2010). Furthermore, as *in vivo* mouse genetic lineage tracing shows, definitive hematopoiesis that occur within the aortic-gonado-mesonephros (AGM) region is dependent on ECs (Zovein et al., 2008; Zhou et al., 2016a); consistently, HSCs originate from the mesoderm as long as the latter retain the potential to differentiate into ECs (Zovein et al., 2008). While embryonic primordial endothelial cells are known to be CD31$^+$ CD41$^-$ CD45$^-$ CD117/c-Kit$^-$ Flk-1$^+$ and lack side population (SP) properties, hemogenic endothelial cells are c-Kit$^+$ CD31$^+$ CD41$^{+/-}$ CD45$^-$ Flk-1$^+$ and have SP properties (Marcelo et al., 2013). In the E11 aorta, hemogenic endothelial cells differentiate into pre-HSCs, which continue to express several endothelial markers (CD31, CD105, Cdh5, Tie1 and vWF) and are CD201/Procr$^+$ (Baron et al., 2018). Recent evidence suggests that hemogenic ECs might eventually persist into adulthood, at least in the bone marrow of chickens (Yvernogeau et al., 2019).

3. Adult endothelial progenitor cells and circulating endothelial cells

Many reports have clearly shown that ECs/EPCs circulate through the peripheral blood. For example, George et al. (1991) detected CECs, by flow-cytometry, in a human vascular injury model. In parallel, other investigators found a minimal detectable amount of ECs of 0.06 ± 0,057 cells/mL in whole blood of patients subjected to catheterization (Sbarbati et al., 1991). Asahara et al. (1999) showed contribution of bone marrow ECs/EPCs with ECs that were localized at distant tissues. In this study, the authors injected bone marrow mononuclear cells, obtained from mice that constitutively express β-galactosidase under the transcriptional regulation of Flk-1 or Tie-2, through the tail vein of immunodeficient mice. Four weeks later, they subjected the transplanted mice to models of endometrial-remodeling, tumor implantation, wound healing, and hindlimb and myocardial ischemia. Some of the β-galactosidase positive cells were found embedded in the blood vessel walls of the affected tissues.

To investigate a possible **bone marrow origin** of adult CECs in humans, Lin et al. (2000) analyzed blood samples from 4 bone marrow

transplant adult recipients who had received gender-mismatch transplants. They were able to expand ECs from blood samples in a culture medium supporting microvascular endothelial growth (endothelial growth medium; EGM). Interestingly, 5% of CECs originated from the donor; however, this subpopulation had much higher expansion (outgrowth) capacity over time *in vitro*. This evidence would suggest that circulating adult angioblasts are located in the bone marrow. The authors also concluded that most of CECs (95%) derived from the vessel walls and/or the persistent recipient bone marrow cells. However, recent articles contrasted these results using similar experimental strategies. These new studies, on the contrary, suggest that EPCs, with *in vitro* outgrowth capacity, would originate mainly from cells present in the host vessel walls and would not derive from the transplanted bone marrow (Fujisawa et al., 2019; Tura et al., 2013).

In the field, **two main types of human circulating EPCs** were traditionally described: early and late EPCs, distinguished by their time of appearance in cultures (Asahara et al., 1997; Hur et al., 2004; Yoon et al., 2005). Such cultures were obtained by seeding blood mononuclear cell samples in plastic coated with fibronectin, collagen-1 o gelatin and after incubation in EGM conditions. While early EPCs have limited expandability *in vitro*, late EPCs can be long-term expanded. The late EPCs were called outgrowth endothelial cells (OECs), due to their properties as endothelial colony-forming cells (ECFCs). OECs differ from early EPCs in that they have increased telomerase activity and an increased ability to incorporate into an endothelial cells monolayer and to form capillary tubes (Hur et al., 2004; Yoon et al., 2005). Hurr et al. (Hur et al., 2004) found that OECs express much higher levels of Cdh5, Flk-1, KDR and MMP2 than early EPCs. Furthermore, by microarray analysis, Medina et al. (2010) showed that early EPCs have an expression profile very similar to monocytes, while OECs correspond more to that of endothelial cells. Furthermore, it was reported that some EPCs show characteristic functions of monocytes, such as antigen presentation and T-cell-co-stimulatory activities (Raemer et al., 2009). Early EPCs were found to include a subpopulation of $CD14^+$ monocyte-derived cells, whereas OECs were almost completely devoid of $CD14^+$ cells (Yoon et al., 2005). The phenotype of early EPCs could eventually result from cell fusion. In fact, the fusion of myelomonocytic and/or macrophages with other cell types is a well-known mechanism in tissue homeostasis and/or regeneration (Willenbring et al., 2004; Camargo et al., 2003, 2004). Alternatively, some endothelial markers contained in platelet vesicles can be transferred to monocytes *in vitro* (Prokopi et al., 2009). Therefore, the fusion of ECs or EC/EPC/platelet cytoplasm transfer with/to monocytes/macrophages, *in vitro* and/or within the bone marrow microenvironment, could partially explain hematopoietic-like properties in some ECs/EPCs (Fig. 1). Consistent with the expected occurrence of these types of events, in a scRNA-seq study focused on the

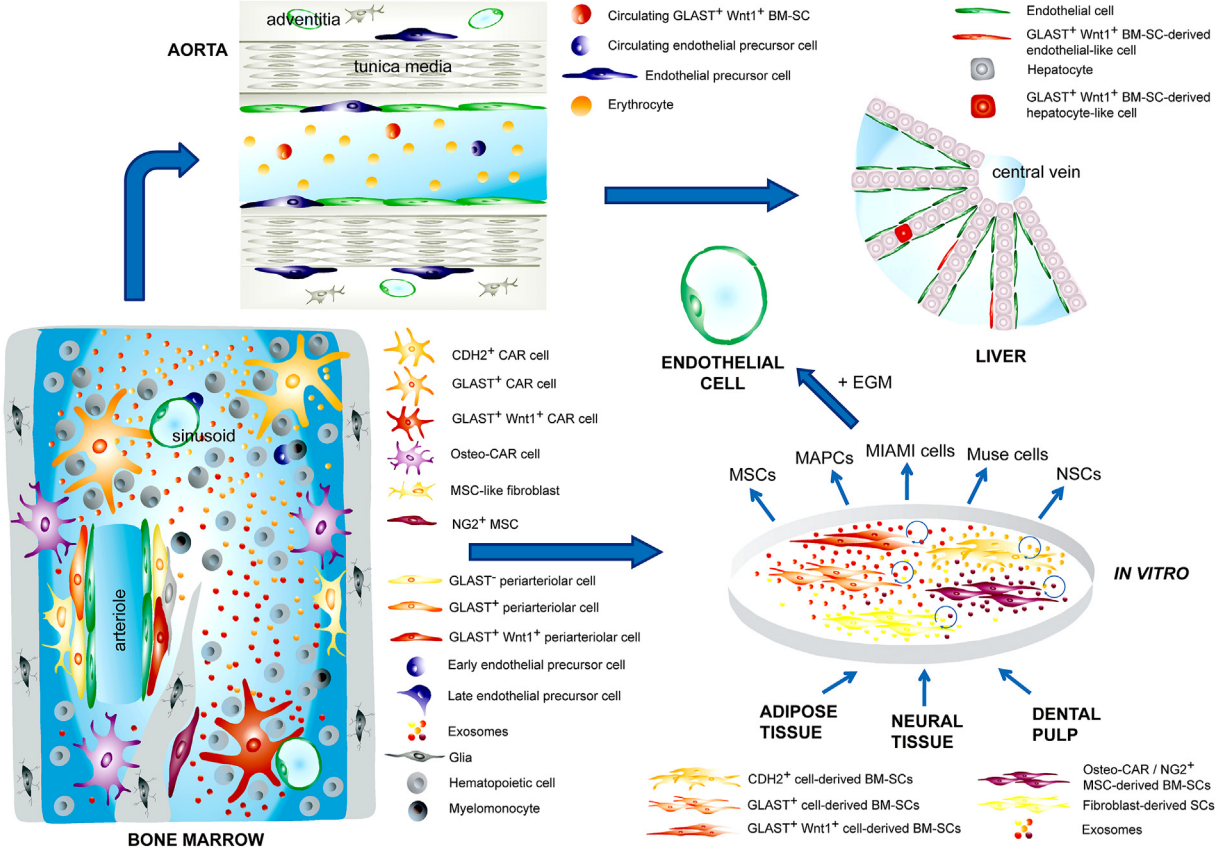

Fig. 1. *Summary Figure.* BONE MARROW: different subpopulations of mouse adult bone marrow stromal cells, which have their counterparts in humans, can contribute with MSCs. IN VITRO: Some of these cells acquire extended plasticity/pluripotent-like properties when incubated in certain environmental conditions, eventually acquiring an ELC differentiation capability. Other reports, showing data from experiments mainly performed *in vitro*, also suggest that adipose, cardiac, neural and dental pulp tissues can be alternative sources of ELCs. AORTA: most of the newly generated adult ECs originate from tissue resident ECs/EPCs; however, circulating ECs (blue), $GLAST^+$ $Wnt1^+$ BM-SCs ($GLAST^+$ $Wnt1^+$ BM-SCs; red), mostly originated from the bone marrow, could to a minor extent also contribute with ECs/ELCs. LIVER: $GLAST^+$ $Wnt1^+$ BM-SCs were seen to be recruited to this organ and to contribute with hepatocyte-like cells (HLCs) and endothelial-like cells (ELCs), *in vivo*. Within the bone marrow and on *in vitro* conditions, hypoxia would favor an increase in microvesicles interchange and cell fusion events among cells as well as some metabolic features, which might be involved in the maintenance of extended developmental plasticity properties in some cell types. In addition, hypoxia could also enhance cell fusion events facilitating the appearance of heterokaryons of myelomonocytes fused with ECs/EPCs, or the fusion of platelets with myelomonocytes, features which can originate a subpopulation of the cells which were formerly named as early EPCs.

stromal fraction of the bone marrow *in vivo*, Baryawno et al. (2019) removed a small cluster of cells that co-express hematopoietic and stromal markers from the dataset, considering them as possible cell doublets.

In the adult, many recent reports suggest that adult **ECs originate primarily from resident vascular ECs/EPCs** rather than from CECs (Fig. 1). For example, by combining adult mice lineage tracing strategies and clonal analyses in a fingertip regeneration model, Rinkevich et al. (2011) observed that, after injury, angiogenesis depends on resident Cdh5$^+$ cells, with no significant contribution of other progenitors and/or circulating cells. The discovery of new markers expressed in ECs subpopulations made it possible to establish a hierarchical relationship between these cells. By mouse genetic lineage-tracing and clonal *in vitro* experiments, Fang et al. (2012) found long-term ECFC properties only in mouse **c-Kit** $^+$ CD31$^+$ CD105$^+$ Sca-1$^+$ ECs. *In vivo*, such cellular fraction formed functional vessels, which were perfused by blood, within implanted Matrigel plugs. These c-Kit$^+$ cells were localized in mouse capillaries, arteries and veins, as well as in human tumor vessels. By immunohistochemistry, it was observed by them that mouse c-Kit$^+$ ECs constituted 18 ± 12% of the liver and 2 ± 1% of the kidney CD31$^+$ ECs, and were also found in lung and subcutaneous vessels. In another study, by applying single cell RNA-sequencing (scRNA-seq) techniques and mouse *in vivo* genetic tracing approaches, Deng et al. (2020) were recently able to demonstrate that c-Kit$^+$ vascular progenitor cells contribute to endothelial repair in the aorta, without a significant contribution from the bone marrow. The authors report that such resident c-Kit$^+$ EPCs would be located in the adventitia or, more rarely, within the intimal layer of the vessel, and that their contribution with new ECs increases in the setting of severe vessel injury. Furthermore, their results also suggest that other subpopulations of vascular EPCs and c-Kit$^-$ ECs would also participate in both aorta angiogenesis and intimal endothelial layer regeneration, in a transplant arteriosclerosis model. Differentiation of c-Kit$^+$ vascular progenitors in ECs was found to require metabolic reprogramming, involving glycolysis and tricarboxylic acid (TCA) cycles (Deng et al., 2020). Consistent with the evidence shown in the previous article, Zengin et al. (2006) described an enrichment of EPCs in deep regions of the human internal thoracic artery wall: in between the muscle (tunica media) and the adventitia vessel layers.

In cases of non-severe lesions and/or under physiological conditions, it was shown that EC turn-over in different mouse tissues depends on local subpopulations of **c-Kit$^-$ ECs/EPCs** (Kusumbe et al., 2014; Yu et al., 2016; Naito et al., 2016; Wakabayashi et al., 2018), further increasing ECs heterogeneity. Thus, Yu et al. (2016) found that c-Kit$^-$ Protein C receptor$^+$ Sca-1$^+$ ECs would constitute the main cell population involved in angiogenesis of the mammary gland *in vivo*. These cells are clonogenic *in vitro*, and it was shown that they originate ECs and pericytes *in vivo* and *in vitro*. Furthermore, the authors also found that this fraction of c-Kit$^-$ ECs also contribute with ECs and pericytes in the skin. They are also involved in the development of retinal blood vasculature development and in vascular repair after ischemic injury to the hindlimbs. Consistently, freshly isolated c-Kit$^-$ Protein C receptor$^+$ Sca1$^+$ ECs expressed higher levels of markers of angiogenesis, vascular development, and endothelial-to-mesenchymal transition than Protein C receptor$^-$ ECs.

According to Naito et al. (2016), the lungs of mice contain a small fraction of resident CD31$^+$ CD45$^-$ ECs with a SP phenotype and *in vitro* ECFCs properties. These SP ECs (**E-SP**) cells are c-Kit$^-$ CD34$^+$ CD44$^-$ Cdh5$^+$ Ephrin B2^{low} Flk-1$^+$ Glycam1$^-$ Hey1^{low} Hey2^{low} Sca1$^+$. By *in vivo* lineage tracing, lung E-SP cells were found not to be derived from the bone marrow. By flow cytometry of fresh samples, it was shown that these cells contribute with lung tumor angiogenesis, since the percentage of E-SP cells increased in this context, from 0.67% (control) to 2.81 and 7.02%, depending on the tumor model. More recently, Wakabayashi et al. (2018) analyzed the signature of mouse liver E-SP cells and found enrichment for CD157/Bst1 and CD200/OX2 transcripts, while CD157$^-$ C200$^-$ ECs almost completely lacked a SP phenotype. The CD157$^+$ C200$^+$ ECs represented 3.9% ± 0.7% of the total liver ECs traced with Cdh5. CD157$^+$ ECs are located in the intimal layer of large blood vessels and were also found in other tissues, including the brain, heart, limb muscles, lungs, retina and skin. However, CD157$^+$ ECs were not found in the capillaries of the heart, liver, and lungs. By mouse genetic lineage tracing, a hematopoietic or bone marrow origin of the CD157$^+$ C200$^+$ ECs was excluded. In addition, these cells were found to play the major role in liver angiogenesis, both in a vascular injury model and in the context of physiological turnover. In such contexts, CD157$^+$ ECs integrated into hepatic capillaries and exhibited morphological and functional properties identical to sinusoidal cells. Moreover, in single cell transplantation experiments, CD157$^+$ C200$^+$ ECs showed self-renewal capacity and integrated into the wall of small and large vessels after liver injury. Furthermore, when transplanted into mice previously subjected to hindlimb ischemia, these cells were recruited into blood vessels and contributed both ECs and αSMA$^+$ pericytes. Finally, after being grafted into a mouse model of hemophilia A, CD157$^+$ C200$^+$ ECs were integrated into the wall of hepatic vessels and rescued both the plasma FVIII activity and the haemophilic bleeding phenotypes.

4. ¿Are there pluripotent-like cells contributing with endothelial cells in the adult?

As mentioned above, microvascular renewal/remodeling over time and/or after injury appears to be highly dependent on tissue resident ECs/EPCs. Therefore, it is likely that CECs derived from distant tissues play a minor role in such processes. Interestingly, it was also shown that some **progenitor cells, lacking an EC/EPC phenotype**, obtained from different tissues but mainly from bone marrow, have the potential to contribute ELCs (Table 1). In most cases, these cells, with extended plasticity (capacity of cells committed to specific lineages to change their fate when exposed to a different microenvironment), were obtained from MSCs cultures under specific *in vitro* conditions. However, their identities *in vivo* remain largely unknown. Interestingly, extended plasticity properties of some types of adult stromal cells, from the bone marrow or other tissues, were confirmed by different groups, since, for example, their ability to differentiate into hepatocyte-like cells (HLCs) was demonstrated, *in vitro* (Fiore et al., 2015; Li et al., 2018; Bao et al., 2016; Piryaei et al., 2011) and *in vivo* (Sierra et al., 2020). Such HLCs do not appear to play a significant role in liver repair; however, these hepatogenic progenitors could be applied in the future in tissue replacement strategies.

In 2002, Jiang et al. (2002) published that mouse bone marrow cells could acquire some properties in common with pluripotent cells (expression of Oct4 and the ability to differentiate into cells of all germ layers) *in vitro*, which they termed multipotent adult progenitor cells (**MAPCs**). However, these cells are negative for Sox2, Nanog and other pluripotent-like markers (Ulloa-Montoya et al., 2007). In addition, the authors suggested that after injection into early blastocysts, individual MAPCs would contribute most somatic cell types (Jiang et al., 2002); however, such experiments/results were not replicated by other groups. Therefore, it would be more correct to state that these cells *in vitro* would have a wide plasticity rather than they could be pluripotent. In fact, several recent scRNA-seq studies found no clear evidence of any pluripotent cells within the bone marrow in the adult (Baryawno et al., 2019; Baccin et al., 2020; Tikhonova et al., 2019). In their protocol, bone marrow mononuclear cells were expanded through various *in vitro* passages as MSCs, at low densities, on surfaces coated with extracellular matrix, and incubated in culture medium supplemented with growth factors, including EGF and PDGF-BB. Regarding the differentiation potential of MAPCs in ELCs, when human AC133$^+$ CD34$^-$ Cdh5$^-$ Flk-1$^+$ MAPCs were cultured with VEGF, they acquired a phenotype of CD34$^+$ Cdh5$^+$ Flk-1$^+$ Tek$^+$ Tie$^+$ vWF$^+$ ELCs (Reyes et al., 2002). Under these conditions, these cells formed capillary-network structures and to uptook DiI-Ac-LDL *in vitro*, characteristic properties of ECs. Furthermore, MAPCs were able to contribute ECs during tumor and wound healing angiogenesis *in vivo*. In another study, it was demonstrated that incubation of mouse MAPCs with VEGF on a fibronectin-coated surface induced their differentiation into Cdh5$^+$ eNOS$^+$ Flk-1$^+$ vWF$^+$ ELCs, with the

Table 1
Pluripotent-like cells as sources of ELCs. Underline phrases denote protocols or tissues used in which ELCs were obtained.

References	Name of cells	Protocol	Tissue sources	*In vitro* differentiation into ELCs	*In vivo* differentiation into ELCs
(Jiang et al., 2002; Reyes et al., 2002; Ulloa-Montoya et al., 2007; Xu et al., 2008; Mahapatra et al., 2010; Ryu et al., 2013; Zhang et al., 2020)	MAPCs	Cells expanded as MSCs for long-term at low density on extracellular matrix in EGF and PDGF-BB ± low O_2	-Adult human, mouse, ovine, rat and swine bone marrow -Swine peripheral blood	Acquisition of EC markers, capacity to uptake Dil-Ac-LDL and capillary network formation, when incubated in angiogenic conditions	Contribution with ELCs when pre-treated *in vitro* under angiogenic conditions
(D'Ippolito et al., 2004; Rahnemai-Azar et al. (2011); Grau-Monge et al. (2017))	MIAMI	Cells expanded as MSCs at low density on fibronectin at low O_2 with 2% FBS	-Adult human bone marrow	Acquisition of EC markers and capillary network formation, when incubated in angiogenic conditions	Contribution with ELCs, even without requirement of *in vitro* pre-treatment
(Kucia et al. (2006); Guerin et al., 2015; Guerin et al. (2017))	VSELs	*In vivo* isolated CD45$^-$ CD133+ lin- Sca1+ cells	-Adult human and mouse bone marrow -Adult mouse uterus -Human cord blood	More evidences are required	More evidences are required
(Kuroda et al. (2010); Gimeno et al., 2017; Uchida et al. (2017); Hosoyama et al. (2018); Tanaka et al., 2018; Yamada et al., 2018; Sun et al., 2020)	Muse	-MSCs cultures subjected to stress conditions -*In vivo* isolated SSEA-3+ human cells -SSEA-3+ FACS-sorted cells from MSCs cultures	-Adult human and rat bone marrow -Adult human adipose tissue	Acquisition of EC markers and capillary network formation, when incubated in angiogenic conditions	Contribution with ELCs even without requirement of *in vitro* pre-treatment

acquisition of a cobblestone EC-like morphology and the ability to uptake Dil-Ac-LDL (Xu et al., 2008). However, when naïve human MAPCs were transplanted into a mouse model of limb ischemia, these cells did not differentiate into ELCs (Ryu et al., 2013). Recently, Zhang et al. (2020) incubated mouse MAPCs with the synthetic apolipoprotein A-I mimetic peptide 5F, together with VEGF, and reported an increase in the expression levels of EC markers, as well as an increase in the formation of vascular tubes and their properties of DiI-Ac-LDL uptake. Finally, Mahapatra et al. (2010) incubated rat MAPCs in the presence or absence of 59-aza-29-deoxycytidine (aza-dC; a specific DNA methyltransferase) and trichostatin A (TSA; a histone deacetylase inhibitor) with or without VEGF. Consistent with the main role of epigenetic changes in the acquisition of an endothelial-like phenotype by MAPCs *in vitro*, the inhibition of DNMT and HDAC increased the expression levels of endothelial cell markers, in the presence or absence of VEGF, and improved their vascular network formation capacity.

In 2004, D'Ippolito et al. (D'Ippolito et al., 2004) cultured bone marrow cells, obtained from vertebral bodies (T1-L5) of cadaveric male and female donors, on fibronectin and under low oxygen tension, for 14 days. Subsequently, small colonies of adherent cells were expanded in similar conditions at low density, with the addition of 2% fetal bovine serum. With this protocol, the authors were able to obtain cells (termed **MIAMI cells**; which stands for marrow-isolated adult multilineage inducible cells) that express BMPR1B (bone morphogenetic protein receptor type 1B), CD29 (β1-integrin), CD63 (a member of the tetraspanin superfamily), CD81 (another tetraspanin, also expressed in hepatocytes and lymphocytes), CD122 (interleukin-2 receptor subunit beta), CD164 (a sialomucin, frequently expressed in CD34$^+$ cells), cMet (a hepatocyte growth factor receptor) and NTRK3 (neurotrophic receptor tyrosine kinase 3), and were negative for c-Kit, CD34, CD36, CD45 and HLADR. MIAMI cells were also positive for some pluripotency markers and were able to originate cells from of all germ layers. Rahnemai-Azar et al. (2011) differentiated MIAMI cells into CD31$^+$ KDR$^+$ vWF$^+$ ELCs *in vitro*, which formed vascular network structures in culture. Furthermore, when naïve MIAMI cells were injected intramuscularly into a mouse model of hindlimb ischemia, a fraction of them acquired an ELC phenotype. Grau-Monge et al. (2017) grafted MIAMI cells, embedded in a 3D structure of gelatin B nanofibers, into a mouse model of hindlimb ischemia, which resulted in better recovery, caused by reduced infiltration of intermuscular adipose tissue and by regeneration of muscle fibers.

In 2006, Kucia et al. (2006) reported, in mice, the possible existence of a subpopulation of bone marrow cells which were named as very small embryonic like stem cells (**VSELs**). They consisted of a homogenous population of CD45$^-$ CD133$^+$ lin$^-$ Sca-1$^+$ cells, which expressed certain levels of embryonic stem cell markers, such as SSEA-1, Oct-4 and Nanog, and showed telomerase activity (Kucia et al., 2006; Ratajczak et al., 2013). These cells could also be differentiated into cells of the various germ layers *in vitro*. Later, these VSELs were found in the peripheral blood, with an increase in this compartment under stress conditions (Kucia et al., 2008). Guerin et al. (2017) injected intramuscularly a combination of sorted human CD45$^-$ CD133$^+$ lin$^-$ VSELs, previously obtained from patients with critical limb ischemia and incubated with EGM, and EPCs, in a mouse hindlimb ischemia model, resulting in a better recovery. The same research group also showed that, under angiogenic conditions *in vitro*, such VSELs acquire a fibroblastic appearance and a cytokine production pattern similar to OECs (Guerin et al., 2015). When human VSELs were embedded in Matrigel and then grafted into a mouse model of hindlimb ischemia, some ECs were stained with an antibody against human CD31 (Guerin et al., 2015). Based on that, the authors suggested that VSELs could contribute to ELCs *in vivo*. However, new evidences must be provided to conclude that VSELs could be differentiated into ECs. Furthermore, some publications even questioned the existence of these VSELs as pluripotent/stem cells (Danova-Alt et al., 2012; Szade et al., 2013; Miyanishi et al., 2013). For example, Miyanishi et al. (2013), using FACS, fractionated mouse bone marrow cell populations according to their expression or not of CD45, lin and Sca-1. The percentage of diploid CD45$^{-/int}$ Lin$^-$ SCA-1$^+$ cells, measured by Syto16 staining, was found to be low (approximately 10%). These authors found few very small cells among VSELs candidates. They also did not find that the candidate cells for VSELs expressed Oct4 or behaved like pluripotent cells. In response, Ratajczak and collaborators argued that the strategy used in these articles to isolate VSELs was inappropriate and different from that employed by them (Suszynska et al., 2014; Ratajczak et al., 2014), and they emphasized that more than 20 independent research groups were able to isolate VSELs and differentiate them into cells corresponding to lineages of different embryonic layers (Ratajczak et al., 2019). Many scientists in the stem cells field remain unconvinced with current data regarding VSELs (Nicholls, 2013; Abbott, 2013). Until now, it has not been possible to obtain 3D structures from expanded cultures of VSELs or the growth of teratomas in immunocompromised mice from these cells, which could eventually confirm a pluripotent state.

In 2010, Kuroda et al. (2010) found that stress conditions *in vitro* would be capable of transforming MSCs from adult human bone marrow into cells with some pluripotent-like properties, which they termed **Muse**

cells (for multilineage differentiating stress enduring cells). These cells lack expression of c-Kit, CD31, CD34 and vWF. In freshly isolated samples, they were found to express SSEA-3 and CD105 (Kuroda et al., 2010), and they were reported to circulate in low numbers through peripheral blood (Tanaka et al., 2018). After an acute human myocardial infarction, the frequency of mobilized bone marrow Muse cells was found to be increased (Tanaka et al., 2018), due to chemoattractant signals, including S1P (Yamada et al., 2018). Muse cells were also isolated from human adipose tissue (Gimeno et al., 2017) and rat BM-MSCs cultures (Sun et al., 2020). In rats, Muse cells were found to co-express SSEA-3 and SSEA-1. Uchida et al. (2017) labeled human bone marrow MSCs with GFP, using lentiviral vectors, and obtained GFP^+ $SSEA\text{-}3^+$ Muse cells from these cell cultures by FACS. These Muse cells were injected systemically into Balb/c mice, which had previously undergone adriamycin-mediated nephropathy, and it was found that the transplanted Muse cells contributed primarily to renal glomeruli ELCs. In this tissue, human Muse cells were identified by GFP expression, fluorescence in situ hybridization (FISH) and labeling with an antibody against the human Golgi complex. Their results also suggest that cell fusion with mouse ECs would not be the main mechanism involved. Hosoyama et al. (2018) used a similar approach to obtain GFP^+ $SSEA\text{-}3^+$ Muse cells and established a mouse aortic aneurysm model. Three days after surgery, the area affected by the aortic aneurysm was dissected and explants were established from this tissue *ex vivo*. Immediately afterwards, 10,000 Muse cells were loaded onto the luminal surface of the samples. After 1–3 weeks, the cocultures were fixed and subsequently analyzed by multiphoton laser microscopy. Several images were obtained every 20 μm from the luminal side to the outer side of the explant. Subsequently, the tissue was embedded in OCT and sagittally sectioned and immunostained. After 7 days *ex vivo*, $CD31^+$ $CD34^+$ GFP^+ cells were observed in the luminal surface. And a week later, $\alpha\text{-SMA}^+$ GFP^+ vascular smooth muscle cells were found in the tunica media, which expressed Calponin in the third week. In other animals, three doses of Muse cells (20,000 cells/time, at 0, 7 days and 2 weeks) were applied systemically to mice treated with aortic aneurysm. After 8 weeks, aortic dilation was significantly reduced in mice treated with Muse cells compared to different controls. When the tissue was analyzed, around 20 $CD31^+$ GFP^+ cells/mm^2 were seen, while this number was much lower when MSCs or $SSEA\text{-}3^-$ non-Muse cells were transplanted instead. Furthermore, also many $\alpha\text{-SMA}^+$ GFP^+ cells were observed at deeper layers of the aorta.

The above data suggest that certain bone marrow cells (but also cells that reside in other tissues) could have the ability to differentiate into ELCs, most likely when cultured under angiogenic conditions. However, a possible contamination of ECs/EPCs in cultures cannot be ruled out. In fact, the culture medium conditions used in these studies for cell reprogramming could downregulate specific EC markers, such as CD34 (Hellwig et al., 1997). Therefore, it is required that all $CD31^+$ $Cdh5^+$ resident ECs be excluded before establishing initial cultures, to avoid any possible EC/EPC contribution. Although the *in vivo* contribution of non-resident cells with ECs in different tissues remains a rare event, these sources could eventually be useful for tissue replacement strategies or other therapeutic applications.

5. Bone marrow stromal cells subpopulations

In 1971, Luria et al. (1971) showed evidence in guinea pig that a low number of progenitor stromal cells circulate through the peripheral blood under physiological conditions. Based on the data analyzed in the previous section and taking into account that the bone marrow would be the main source of circulating stromal cells with increased plasticity (Sierra et al., 2020; Hong et al., 2009) it is worth reviewing the recent knowledge on the relevant BM stromal subpopulations.

Bone marrow stromal cells (BM-SCs) include adventitial or reticular cells, most commonly found in proximity/contact with vessels, which are likely the major source of mobilized peripheral blood stromal cells and MSCs cultures (Sierra et al., 2020). Therefore, it is expected that some of these cells show extended plasticity and that they may eventually differentiate into ELCs. These stromal cells are heterogeneous and frequently play a fundamental role in the niches of the HSCs and/or influence their differentiation towards different lineages. Several recent publications addressed the composition of these stromal subpopulations in **mice** (Table 2; Fig. 1). Zhao et al. (2019) described a subgroup of mouse BM-SCs that express **Cdh2/N-cadherin**, preferentially located at endosteal regions, which would have the ability to maintain HSCs that are capable of surviving chemotherapy; whereas the majority of BM-SCs in arteriolar or sinusoidal-enriched areas (described below), would influence HSCs that are sensitive to chemotherapy. Originally, two main subpopulations of mouse bone marrow stromal/reticular cells were described: most of them are found around the sinusoids, while a smaller fraction forms a sheet of cells around arterioles (Sierra et al., 2020; Asada et al., 2017; Kunisaki et al., 2013). Both **perisinusoidal** and **periarteriolar stromal cells** produce and release different factors that, in combination, are necessary for the maintenance of HSC (Asada et al., 2017; Baccin et al., 2020). Perisinusoidal stromal cells express Cxcl12 (called CAR cells, for Cxcl12 abundant reticular cells), LepR, and low GFP levels in $Nestin^{GFP}$ mice ($Nestin^{dim}$), and lack the expression of α-SMA and Myh11 (Asada et al., 2017; Kunisaki et al., 2013). The periarteriolar stromal cells are, on the other hand, $\alpha\text{-SMA}^+$ $LepR^{-/low}$ $Myh11^+$ and $Nestin^{bright}$ (Asada et al., 2017; Kunisaki et al., 2013; Baryawno et al., 2019). Both cell types are traced in $NG2^{Cre}$;$R26R^{Tom}$ and SCF^{GFP} mice, although NG2/Cspg4 expression levels are much higher in periarteriolar stromal cells (Asada et al., 2017).

By **scRNA-seq analyses**, different research groups recently discovered great heterogeneity among bone marrow stromal populations (Baryawno et al., 2019; Baccin et al., 2020; Tikhonova et al., 2019). After analyzing 25933 cells, Baryawno et al. (2019) were able to characterize gene signatures from 17 distinct subpopulations of bone marrow stromal cell in 6–8 week-old C57BL/6 males. As working material, they used pooled samples that included both bone marrow and bone compartments. Among the stromal cells capable of contributing MSCs, they were able to identify: 1) LepR stromal cells (which they named as LepR MSCs and probably overlap with CAR cells); 2) two subsets of fibroblasts, displaying MSC markers, and 3) pericytes (expressing α-SMA and probably overlapping with periarteriolar stromal cells). As the authors were unable to locate the different subpopulations within the tissue, fibroblasts with MSC-like characteristics could eventually reside outside the bone marrow.

Baryawno et al. (2019) were able to distinguish at least 4 different clusters of LepR stromal cells, with variations between them in the expression levels of several markers including Cxcl12, LepR and Grem1. Among them, a subgroup (#4) expressed higher levels of osteolineage genes (for instance, Alpl, Mmp13, osterix/Sp7 and Wif1). However, such heterogeneity could be even greater, since some LepR cells registered high levels of expression of CD44, Eng, Il6, Jag1, Ng2 and/or Nt5e. LepR stromal cells were found to express significant levels of Pdgfra (a marker absent in pericytes), cadherin 11 and N-cadherin. Regarding these specific markers, Pdgfra is associated with the delamination of neural crest cells (Soldatov et al., 2019), while cadherin 11 and N-cadherin play important roles in the migration of the same embryonic population (Blaue et al., 2018; Xu et al., 2001). Interestingly, it has recently been shown that PDGFRα activation by cadherin-11 signaling promotes fibroblast proliferation (Madarampalli et al., 2019). Strikingly, LepR stromal cells expressed most of the examined transcriptional regulatory factors described for neural crest cells (88% vs. 64%; cluster 1 -LepR stromal cells-vs. cluster 12 -including all pericytes-). Such genes were associated with different functions of the neural crest: induction and/or delamination ($Dlx5^{low}$, $Dlx6^{low}$, Heyl, Irx5, $Klf6^{high}$, Junb, Maf, $Mafb^{low}$ and $Zeb2^{high}$); specific markers (Id3, Id4, Jun^{high} and $Six1^{high}$); ectomesenchymal markers ($Foxc1^{high}$, $Meis3^{high}$, Prrx1, Runx2 and Sox4); differentiation and repression of Snai1 ($Snai2^{high}$), or of derivatives (Egr1, FoxS1 and $Runx1^{high}$) (Baryawno et al., 2019; Soldatov et al., 2019; Kee and Bronner-Fraser, 2001; Li et al., 2019; Balakrishnan et al., 2016;

Table 2

Expression profiles of different BM-SCs. Gene signatures, obtained by RNAseq, scRNAseq or microarray studies, regarding human or mouse cell subpopulations which would likely contribute *in vitro* with MSCs cultures, are shown. In between all reported genes, those that were analyzed in common in different publications or were considered most relevant for characterizing each phenotype, were selected to be herein included.

Source	Species / Condition / Technique	Mouse lines	Method of isolation	Cell type	Markers (-2 -1 0 1 2)
Asada et al., 2017	Mouse / in vivo / RNAseq	$Lepr^{Cre}$;$R26^{Tom}$ $NG2^{Cre}$;$R26^{Tom}$ $Myh11^{cre}$;$R26^{Tom}$	Flushed BM	Perisinusoidal cells Perisinusoidal + periarteriolar cells Periarteriolar cells	Abbc9 Acta2 Adipoq Alpl Angpt1 Anpep Cxcl12 Des Dl1 Dlk1 Fzd8 Gja1 Il7 Jag1 Jag2 Kcnj8 Kitl Mcam Ng2 Ogn Pdgfra Sele Spp1 Rgs5 Thpo Vcam1
Baryawno et al., 2019	Mouse / in vivo / scRNAseq	C57BL/6 mice (CD45.2, Jackson Lab)	Pooled samples; enzymatically digested bone fragments and flushed BM	Fibroblasts-1 Fibroblasts-2 Lepr MSCs Pericytes	Acta2 Adipoq Alpl Bglap Cdh2 Cdh11 Cebpa Cxcl12 Des Esm1 Foxc1 Gja1 Kitl Lepr Lpl Ly6a Mcam Mef2c Ng2 Pdgfra Runx2 Sfrp4 Sp7 Spp1 Vcam1 Vdr Vegfa
Ilas et al., 2020	Human / in vivo / microarray	Femur head from patients with femur fracture	Trabecular bones, enzymatically dissociated. BM-MNCs were FACS sorted	CD56+ CD146-CD271+ CD56- CD146-CD271+ CD56- CD146+CD271+	Acta2 Alpl Angpt1 Bglap Cdh11 Cebpa Col1a1 Cxcl12 Fabp4 Fzd8 Gja1 Il7 Jag1 Lepr Lpl Mcam Myh9 Nes Ng2 Pdgfra Runx2 Sfrp4 Sp7 Spp1 Vegfa Wif1
Tikhonova et al., 2019	Mouse / in vivo/RNAseq	$Lepr^{Cre}$;$R26^{Tom}$	Flushed BM	Lepr cells	Adipoq Alpl Angpt1 Bglap Cebpa Ccl19 Cdh5 Col1a1 Col2a1 Cxcl12 Egr1 Foxc1 Gas6 Il7 Kitl Lepr Mcam Mfap4 Pdgfra Runx2 Sele Sparc Vcam1 Vdr
Tikhonova et al., 2019	Mouse / in vivo / scRNAseq (traced cells in mouse lines)	$Lepr^{Cre}$;$R26^{Tom}$	Flushed BM	Lepr adipogenic-primed Lepr osteo-primed (P3) Lepr osteo-primed (P4)	Adipoq Alpl Bglap Cd302 Chrdl1 Cldn10 Col1a1 Cxcl12 Esm1 Foxc1 Gas6 Lpl Mef2c Mgp Mmp13 Prelp Runx2 Serping1 Sfrp4 Sp7 Spp1 Timp1 Vcam1 Wif1
Zhao et al., 2019	Mouse / in vivo / RNAseq	$Ncad^{Tdt}$(bone) $Ncad^{Tdt}$(BM) $Lepr^{Cre}$;$R26^{Tom}$ $Cxcl12^{dFP}$ $Nestin^{GFP}$	Enzymatic digestion. Flushed BM	Bone and BM stromal BM stromal/ perivascular cells	Bglap Cdh2 Col1a1 Col2a1 Cxcl12 Dmp1 Fgf23 Gla Gli Itgav Itgb1 Kitl Lepr Ly6a Mcam Nes Ng2 Nt5e Pdgfra Pf4 Prrx1 Runx2 Sele Slamf1 Sp7 Spp1 Vcam1

Marmigere et al., 2006; Shirai et al., 2019; Heglind et al., 2005). These properties could suggest that some LepR MSCs have the ability to proliferate and mobilize into the peripheral blood, in response to systemic signals, an issue that has not yet been addressed. Finally, LepR stromal cells also expressed Hif-1α, which plays an important role in hypoxic conditions (Baryawno et al., 2019).

Wolock et al. (2019) analyzed 2847 BM-SCs using scRNA-seq. Long bone samples were collected from 8 to 16-week-old C57Bl/6 mice which were subsequently treated with collagenase-dispase. The fraction of $CD45^-$ $Ter119^-$ $CD31^-$ cells were sorted out, and from the dataset those corresponding to putative doublets and contaminating hematopoietic and endothelial cells were removed. Seven clusters could be identified, including MSCs (the most abundant fraction), preadipocytes, pro-osteoblasts and pro-chondrocytes. Using SPRING the authors were able to show a continuum of cell states distributed in two major branches, suggesting a steady-state differentiation process. Both branches were bridged by MSCs, a cluster enriched in the expression of Adipoq, Cb1n1, Cxcl12, FoxC1, Kitl, Klf15, LepR, Lpl, Nt5e, Pparg, Prrx1, Runx1, Runx2 and Snai2. Such markers are more highly expressed in non-osteogenic biased LepR cells (Table 2). It is worth noting that Velocity analyses of transcriptional trajectories identified MSCs as the strongest source of other stromal cell types.

Also by scRNA-seq, Tikhonova et al. (2019) analyzed 6617 cells, which they collected from different mouse Cre lines (Cdh5, LepR and Col1a1), of either sex, which were intraperitoneally injected with tamoxifen 1–3 months earlier. With such studies, their objective was to analyze differences in the expression profile between bone marrow vascular endothelial cells, LepR stromal cells and osteolineage cells, respectively. The samples were obtained by bone marrow flushing, without applying enzymatic procedures. Among the LepR cells, they were able to distinguish 3 different groups: one adipogenic-primed and two osteogenic-primed (Table 2). Most HSC niche factors were found upregulated within adipogenic-primed LepR cells, which most often occupy a perisinusoidal territory (Baccin et al., 2020). Only osteogenic-primed LepR expressed Runx2, and could partially overlap with cells described by Yang et al. (2017) as sitting at the top of the bone marrow stem cell hierarchy. The authors included data on the expression levels of the main genes that characterize each of three cellular subpopulations in each mouse analyzed. With these data we were able to calculate the mean optic density and standard deviation values for each gene. With regard to specific endothelial cell markers, excluding Vcam1 (a known LepR marker), the expression profile of LepR cells showed greater heterogeneity compared to Col1a1 cells (standard deviation values: 0.41 vs. 0.32; cells traced with LepR vs. cells traced with Col1a1; $p = 0.033$; t-student). These results suggest a greater heterogeneity than expected and/or an especial epigenetic state, in subpopulations of LepR-traced cells.

More recently, Baccin et al. (2020) analyzed bone marrow cells from 8- to 12-week-old C56Bl/6J females. During sample collection, they first obtained a cellular suspension directly after crushing femurs, tibiae, hips and ribs: which was classified as corresponding to the bone marrow fraction. Subsequently, they enzymatically treated bone fragments and thus obtained a second fraction, classified as bone-associated cells. In addition to scRNA-seq, they also performed spatially resolved transcriptomics (by laser microdissection of specific areas of the bone marrow and subsequent RNAseq of the different areas) of each of the two previously described fractions. They analyzed 7497 cells, including hematopoietic cells. In addition, in order to analyze sparsely represented stromal subpopulations, $CD45^+$ and/or $CD71^+$ populations were excluded in some of the comparisons. Interestingly, they were able to separate two CAR populations ($Alpl^-$ $Cxcl12^+$ adipo-CAR, and $Alpl^+$ $Cxcl12^+$ osteo-CAR) that probably overlap with adipogenic-primed and osteogenic-primed LepR clusters described by Tikhonova et al. (2019), respectively. In this study, adipo-CAR cells were preferentially located in areas with greater abundance of sinusoids, while osteo-CAR cells were found mainly in arteriolar and non-vascular niches. Furthermore, a new population of $Ng2^+$ MSCs was described. These cells were $Lepr^-$ $Nestin^+$ and were located mainly at endosteal areas. They had an osteogenic-like phenotype and were characterized by high levels of expression of CD61/Itgb3 and CD200. By means of pseudo-time gene expression analysis using RNA-Velocity, $Ng2^+$ MSCs occupied the top of the developmental hierarchy of bone marrow stromal cell, being able to originate osteoblasts, adipo-CAR cells, osteo-CAR cells, chondrocytes and some fibroblasts. Moreover, their data suggest high heterogeneity in

adipo-CAR and Ng2$^+$ MSCs clusters, which would require further analyses with a higher number of sorted CD45$^-$ CD71$^-$ Lin$^-$ VCAM-1$^+$ cells. New studies could help elucidate which cells have the greatest developmental potential, as current data differ between studies (Baccin et al., 2020; Wolock et al., 2019). Finally, they described subpopulations of endosteal and arteriolar fibroblast Cd34$^+$ Cd44$^+$ Cd90/Thy1$^+$ Pdgfra$^+$ Sca1$^+$ cells, which could partially overlap with MSC-like fibroblast cells described by Baryawno et al. (2019), as previously discussed (Table 2; Fig. 1). And some of these arteriolar fibroblasts probably correspond to periarteriolar stromal cells (Asada et al., 2017).

Less is known about the stromal subpopulations of **human bone marrow** (Tables 2–4). ScRNA-seq analysis of these stromal cells has not yet been performed. Boiret et al. (2005) obtained bone marrow samples from the cancellous femur head of 40 individuals (66 ± 2 years) that were subsequently mechanically dissociated. These cells were cultured for 1–3 days and, subsequently the CD45$^-$ CD14$^-$ cell fraction was obtained and replated. These cells showed CFU-Fs properties. All sorted cells were CD73$^+$ (Nt-5e) and most of them, CD49a/Itga1$^+$. In the mouse, both markers were expressed mainly in some adipogenic LepR stromal cells and in some bone marrow stromal cells tracked in Nestin-Cre mice (Baryawno et al., 2019; Zhao et al., 2019; Baccin et al., 2020). Matsuoka et al. (2015) immunomagnetically separated CD45$^-$ lin$^-$ bone marrow mononuclear cells into different fractions according to their expression of CD271 (p75NTR, TNFRSF16) and/or SSEA3. All the fractions contained CFU-Fs, with highest numbers in the CD271$^+$ fractions and a very rare incidence in the CD271$^-$ fractions. Other groups consistently reported that CD271$^-$ bone marrow mononuclear cells lack significant CFU-Fs properties (Jones et al., 2002; Quirici et al., 2002; Tormin et al., 2011; Mabuchi et al., 2013). MSCs obtained from CD271$^+$ SSEA$^+$ cells showed an osteogenic-biased expression profile, and lacked adipogenic differentiation capacity (Matsuoka et al., 2015). Such properties could reflect the cellular phenotype *in vivo*, different from that of mouse adipo-CAR/LepR-adipogenic-primed stromal cells. Mabuchi et al. (2013) crushed bone fragments from the femoral head and collected bone marrow cells separately, before and after enzymatic treatment of bone pieces. They found 10-fold higher CFU-F numbers in the **endosteal-associated stromal cell fractions** when compared to non-endosteal-associated stromal cells. Among markers expressed in both fractions, CD90/Thy1$^+$ and CD271$^+$ were the two most expressed in bone-associated cells. When CD90$^+$ cells were assayed for CFU-Fs in limiting dilution assays, the highest clonogenic properties of endosteal cells were obtained within the CD271$^+$ fraction. Other CD90$^+$ fractions analyzed (CD73, CD105, CD106/VCAM-1, MSCA-1 or STRO-1) also rendered CFU-Fs. A subpopulation of CD90$^+$ CD271$^+$ cells, with higher VCAM-1 expression, showed higher proliferation and migratory rates than the others. It is worth noting that in the mouse, VCAM-1 gene is more expressed in adipo-CAR compared to osteo-CAR cells (Tikhonova et al., 2019) (Table 2).

In mice, **CD56/NCAM$^+$ stromal cells** were found enriched in endosteal regions (Zhao et al., 2019). Battula et al. (2009) isolated human bone marrow fractions using antibodies against CD56/NCAM, CD271 and MSCA-1. They reported that a subpopulation of CD56$^+$ CD271$^+$ was found to be enriched in CFU-Fs compared to those CD56$^-$ CD271$^+$. Among CD56$^+$ cells, the authors also found a higher number of clonogenic cells in the MSCA-1$^+$ fraction, compared to those MSCA-1$^-$. In a recent paper, Ilas et al. (Ilas et al. (2020) obtained samples from patients with a femoral neck fracture and collected trabecular bone from the femoral head. After enzymatic dissociation, different subsets of CD45$^-$ CD271$^+$ cells were isolated by fluorescence-activated cell sorting (Table 2). They compared the expression pattern of these subpopulations by microarray and qPCR analysis. They found that CD56$^+$ expressed higher levels of mRNA from osteogenic-related genes (Alpl, Bambi, Bglap, Bmpr1a, Cdh11, Chad, Fgfr1, Fgfr3, Fzd7, Fzd8, Nog, Omd, Pdgfra, Runx2, Sfrp4, Sp7, Spp1, Tnfrsf11b, Twist1 and Wif1) than CD146$^+$ cells (see also Table 3). In contrast, CD146$^+$ cells were enriched in adipogenic-associated genes (Fabp4, Gata2, Pparg). CD56$^-$ CD146$^-$ showed an intermediate phenotype, with a greater general similarity to CD56$^+$ cells, with the exception of some hematopoietic niche factors such as Angpt1 or Cxcl12, which were upregulated. As expected, CD56$^+$ CD271$^+$ were found to be closely associated with the endosteum, while some CD56$^-$ CD271$^+$ were located in deeper areas of the bone parenchyma, consistent with other publications (Tormin et al., 2011; Sacchetti et al., 2007).

In iliac bone marrow aspirates, Sacchetti et al. (2007) only found clonogenic properties among CD146/Mcam$^+$ cells, which consisted of **adventitial reticular cells, surrounding sinusoids**. More recently, Tormin et al. (2011) found enrichment for CFU-Fs within two fractions of iliac bone marrow aspirates: CD45$^-$ CD146$^+$ CD271$^+$ lin$^-$ and CD45$^-$ CD146$^{-/low}$ CD271$^+$ lin$^-$. CD146$^+$ CD271$^+$ cells were found surrounding

Table 3

Characterization of the phenotype of bone marrow stromal subpopulations according to the expression levels of commonly analyzed genes. Data were obtained from references shown in Table 2. Only those genes characterizing each cell type that were reported in two or more articles are considered.

Bone marrow stromal subpopulations	Markers distributed according to their expression levels			
	High	Intermediate	Low	No/Negative
Human CD56$^+$ CD146-	Bglap, Col1a1, Sfrp4, Spp1, Wif1	Cdh11, Fzd8, Ng2, Pdgfra	Alpl, Col1a2, Cxcl12, Gja1, Runx2, Sp7	Acta2, Angpt1, Il7, Jag1, Lepr, Lpl, Mcam, Nes, Vegfa
Human CD56$^-$ CD146-	Cxcl12	Angpt1, Bglap, Cdh11, Pdgfra, Sfrp4, Wif1	Alpl, Col1a1, Col1a2, Fzd8, Gja1, Il7, Lepr	Acta2, Jag1, Lpl, Mcam, Nes, Ng2, Runx2, Sp7, Spp1, Vegfa
Human CD56$^-$ CD146+	Angpt1, Mcam	Cdh11, Cxcl12, Lpl, Ng2	Alpl, Jag1, Lepr, Nes, Vegfa	Acta2, Bglap, Col1a1, Col1a2, Fzd8, Gja1, Il7, Pdgfra, Runx2, Sfrp4, Sp7, Spp1, Wif1
Mouse bone marrow Cdh2-traced		Cdh2, Col1a1, Cxcl12, Grem1, Kitl, Lepr, Pdgfra, Pdgfrb	Col1a2, Nes, Runx2, Sp7, Vcam1	Bglap, Mcam, Ng2, Spp1
Mouse endosteal Cdh2-traced	Bglap	Cdh2, Col1a1, Cxcl12, Kitl, Lepr, Ng2, Pdgfra, Pdgfrb	Col1a2, Grem1, Mcam, Runx2, Sp7, Spp1, Vcam1	
Mouse LepR adipogenic-primed (adipo-CAR)	Adipoq, Cxcl12, Foxc1, Kitl, Vcam1	Angpt1, Cdh2, Cdh11, Gja1, Grem1, Il7, Lepr, Lpl, Pdgfra, Pdgfrb, Sfrp4, Vegfa	Alpl, Col1a2, Des, Fzd8, Jag1	Acta2, Bglap, Col1a1, Mcam, Mef2c, Mmp13, Nes, Ng2, Runx2, Sp7, Spp1, Wif1
Mouse LepR osteogenic-primed (osteo-CAR)	Alpl, Bglap, Col1a1	Cdh2, Cdh11, Grem1, Kitl, Lepr, Mef2c, Mmp13, Pdgfra, Pdgfrb, Runx2, Sp7, Vegfa, Wif1	Col1a2, Cxcl12, Des, Fzd8, Jag1, Spp1	Acta2, Lpl, Mcam, Nes, Ng2, Sfrp4, Vcam1
Mouse periarteriolar/ pericytes	Acta2, Des	Cdh2, Jag1, Lpl, Mcam, Ng2, Pdgfrb	Cdh11, Cxcl12, Il7, Kitl, Nes	Adipoq, Alpl, Bglap, Foxc1, Gja1, Grem1, Lepr, Mef2c, Pdgfra, Runx2, Sfrp4, Spp1, Vcam1

Table 4
Characterization of different human bone marrow stromal subpopulations. Data from studies in which specific fractions of bone marrow mononuclear cells were selected as initial samples for establishing MSC cultures are herein analyzed, in a temporal-frame order.

References	Sample	Sorted cell population	CFU-F	*In vivo* markers	Observations
Jones et al., 2002	Bone marrow aspirates	CD45^{low} D7-FIB	100x increased when compared to bone marrow mononuclear cells (100x)	CD10$^+$, CD13$^+$, CD34-, CD90$^+$, CD105+, CD133-, CD271+, HLA-DR+, c-Kit- (most of cells), STRO-1+	Multilineage differentiation in MSCs
Quirici et al., 2002	Bone marrow aspirates	CD271+ (90%) CD271-	1 every 370 cells (61x) None	CD34$^+$ (44%), CD90/Thy1+ (6,3%), CD133+ (49%)	Cells with long processes were found in contact with sinus endothelial cells and with many hematopoietic cells. MSCs obtained from this fraction show full differentiation capacity, with higher adipogenic capacity than CD45- glycophorin A- BM-derived MSCs
Boiret et al., 2005	Bone marrow aspirates	CD45- CD14-	Yes	CD73/NT-5e+, CD49a/ITGA1+ (most of cells)	Markers expressed in mouse LepR+ and Nestin + bone marrow stromal cells
Sacchetti et al., 2007	Enzymatically treated bones	CD45- CD146+	1/38 cells (830x)	ALP+, CD31-, CD34-, CD45$^+$ (2%), CD105+	Clonogenic cells were only obtained from CD146+ cells, that were only found near vessels at non-endosteal bone marrow regions
Battula et al., 2009	Femoral shafts of patients undergoing total hip replacement	CD56+ CD271bright CD56- CD271bright	1/13 cells (180x) 1/42 cells (60x)	Non determined	Multilineage differentiation in MSCs Multilineage differentiation in MSCs
Tormin et al., 2011	Iliac bone marrow aspirates	CD45- CD146+ CD271+ lin- CD45- CD146-/low CD271+ lin- CD271-	1/50 cells (400x) 1/24 (833x) None	In the two fractions, cells were mostly positive for ALPL, CD90, CD105, CEBPA, GD2, LPIN1, Nanog, Oct4, PDGFRB, SSEA4 and STRO-1, and around 30% of them were SOX2+	CD271+ CD146+ cells consisted of reticular cells surrounding capillaries and larger vessels, whereas CD271+ CD146- cells were mainly localized at endosteal trabecular bone areas
Mabuchi et al., 2013	Crushed femur head bone fragments and collected bone marrow cells before and after enzymatically treating bone pieces	CD90+ CD271+ CD271-	Higher clonogenic properties than CD90$^+$ cells coexpressing CD73, CD105, MSCA-1, STRO-1 or VCAM-1 None	CD49a, CD49d/ITGA4, CD73, CD140b/PDGFRB, CD146/MCAM, STRO-1, VCAM-1 and MSCA-1/TNAP	Highest clonogenic properties were found within endosteal- associated bone marrow stromal cells
Pinho et al., 2013	Fetal bones (13–20 weeks of gestation)	CD51$^+$ PDGFRα+ CD51+ PDGFRα- CD51- PDGFRα+	1/16 cells 1/50 cells 1/100 cells	ANGPT1+, CD146+, CXCL12+, Nestin+, OPN+, SCF+, VCAM1+, VE-Cadherin-	Multilineage differentiation in MSCs
Matsuoka et al., 2015	Bone marrow aspirates	CD45- lin- CD271+ SSEA3+ CD45- lin- CD271+ SSEA3+ CD45- lin- CD271- SSEA3- CD45- lin- CD271- SSEA3+	1 every 6 cells 1/264 cells 1/13766 cells 1/84231 cells	Non-determined	MSCs obtained from this fraction lacked from adipogenic differentiation capacity: they are likely osteogenic progenitors Multilineage differentiation in MSCs Multilineage differentiation in MSCs Multilineage differentiation in MSCs
Muniz et al., 2015	Bone marrow aspirates	CD13high CD45- CD105+	Yes	CD10$^+$ (89%), CD11b-, CD34-, CD73$^+$ (moderate), CD90$^+$ (heterogeneous levels), CD105low, CD117-, CD146low, CD271, HLA-DRlow (91%), MSCA-1, SSEA-4low, STRO-1 (heterogeneous levels)	CD90 expression levels were reduced in BM reactive cells. Multilineage differentiation in MSCs
Herrmann et al., 2019	Bone marrow aspirates	CD34- CD45- CD146+ CD146+ NG2+ CD34- CD45- CD73$^+$	Yes Yes Yes	Non determined	Multilineage differentiation in MSCs Multilineage differentiation in MSCs Multilineage differentiation in MSCs

sinusoids and larger vessels, while those CD146$^-$ CD271$^+$ were located mainly in areas of endosteal trabecular bone.

Pinho et al. (2013) identified CD51/Itgav$^+$ PDGFR-α$^+$ fetal BM-SCs in endosteal areas or **near α-SMA $^+$ perivascular cells**, the latter probably equivalent to mouse Nestin^{GFP} $^+$ perivascular cells. This cell subpopulation contributed to a large fraction of Nestin $^+$ cells and expressed higher levels of CXCL12 and VCAM-1 compared to CD146$^+$ cells. They were enriched in CFU-Fs and could also be cultured as floating spheres. CD51$^+$ PDGFR-α$^+$ cells were also found in adult bone marrow samples, albeit in very small numbers.

Muniz et al. (2015) reported a subpopulation of CD13^{high} CD45$^-$ CD105$^+$ BM-SCs, with CFU-F properties. More recently, Hermann et al.

(Herrmann et al., 2019) found differences in the incidence of different stromal cell subpopulations (CD34$^-$ CD45$^-$ CD73$^+$, located in endosteal and perivascular and stromal sites; CD34$^-$ CD45$^-$ CD146$^+$, perivascular progenitor cells, and CD146$^+$ NG2$^+$, pericytes) according to their origin (femur head, iliac crest or vertebral body) and they were able to derive MSCs from all these fractions. Functional and scRNA-seq studies, using different subpopulations of human stromal cells, are required to better understand the heterogeneity of this compartment and to compare it with its counterparts in mice.

In summary, current knowledge suggests a greater abundance of osteogenic stromal progenitor cells in the adult human bone marrow compared to the mouse. However, the non-endosteal fraction is probably the major source of hematopoietic niche factors and contains predominantly adipogenic cells in both species. From previous data, we can conclude a great heterogeneity between BM-SCs with CFU-F properties, including endosteal osteogenic-biased progenitor populations, perisinusoidal CAR cells and pericytes/Nestin$^+$ perivascular cells, subpopulations that are largely concordant with some differences between species.

6. GLAST $^+$ Wnt1$^+$ BM-SCs, a source of endothelial cells

Recently, a subpopulation of perisinusoidal and periarteriolar BM-SCs was found to express CD44, desmin^{low}, fibronectin, GLAST (Glutamate-Aspartate Transporter) and vimentin (Sierra et al., 2020). Both types of stromal cells included a small subset (representing nearby 10% of GLAST$^+$ cells) which was traced in Wnt1^{Cre};R26R^{Tom} mice, although they did not originate in the neural crest. In GLAST^{CreERT2};R26R^{Tom} and Wnt1^{Cre};R26R^{Tom} mice, tomato$^+$ (Tom$^+$) perisinusoidal cells exhibited multiple long processes in contact with neighbouring cells, as previously described in humans (Quirici et al., 2002). Furthermore, the BM-SCs traced with Wnt1 were not associated with the endosteum with the exception of few periarteriolar cells. These findings were not inconsistent with previously published data on the expression pattern of GLAST in mouse BM-SCs (Baccin et al., 2020). Moreover, GLAST$^+$ Wnt1$^+$ BM-SCs were shown to have CFU-F properties and to contribute with 10–15% of the total colonies formed (Fig. 1). Besides, Tom$^+$ colonies were exclusively made up of traced cells.

Under physiological conditions, GLAST$^+$ Wnt1$^+$ BM-SCs were found circulating through peripheral blood, at a very low frequency (Sierra et al., 2020). In different liver injury models, GLAST$^+$ Wnt1$^+$ BM-SCs were mobilized to peripheral blood and recruited in the liver, where they contributed ELCs and HLCs. In both bone marrow and peripheral blood mononuclear fractions, GLAST$^+$ Wnt1$^+$ BM-SCs were negative for HSCs, hematopoietic cells and EPCs markers, such as c-Kit, CD11b, CD31, CD45 and Sca-1. Therefore, these and other evidences (Asahara et al., 1999) suggest that, at least in adult mice, some ELCs could originate *in vivo* by vasculogenesis and not only by angiogenesis after injury.

When healthy and fibrotic livers from Nestin^{Cre};R26R^{Tom} mice were analyzed, no ECs or HLCs expressing Tom were observed (Sierra et al., 2020). Taking into account that only periarteriolar and not perisinusoidal stromal cells express Nestin at sufficient levels (Baryawno et al., 2019), these results would exclude periarteriolar stromal cells as an origin of GLAST$^+$ Wnt1$^+$ BM-SCs recruited in the liver. Thus, these stromal cells most likely correspond to a rare subpopulation of LepR cells located in sinusoid-enriched areas of the bone marrow, where adipo-CAR cells predominate. Previously, Wnt1 was previously shown to be activated in a subpopulation of bone marrow cells after myocardial injury, and this feature was found to induce both proliferation of bone marrow mononuclear cells with CFU-GM properties and their invasive behavior towards Cxcl12/SDF1 (Assmus et al., 2012). Unfortunately, the authors did not address the behavior of the BM stromal compartment in this context.

Activation of the Wnt1 promoter could eventually be a hallmark of a small fraction of GLAST$^+$ stromal progenitors, which could gain in the ability to sense and respond to signals released by injured tissues, moving into the bloodstream and then toward the source of those signals. To our knowledge, this is the first *in vivo* report showing a contribution of BM-SCs with ELCs in any distant tissue without involving bone marrow EPCs.

7. Mechanisms involved in enhanced plasticity/pluripotency acquisition of progenitor cells

Other tissues were reported to contain progenitor cells, which do not express EPC markers, with the ability to generate ELCs. These include adipose (Miranville et al., 2004; Planat-Benard et al., 2004; Navarro et al., 2015), neural (Oishi et al., 2004; Wurmser et al., 2004; Ii et al., 2009) and dental (Gronthos et al., 2000; d'Aquino et al., 2007; Marchionni et al., 2009; Aksel and Huang, 2017; Cordeiro et al., 2008; Sakai et al., 2010; Zou et al., 2017; Zou et al., 2019; Sasaki et al., 2020; Iohara et al., 2008) tissues.

The data discussed above suggest that some adult progenitor cells in different tissues would show extended plasticity, and the ability to acquire morphological and functional characteristics of ELCs. Furthermore, some of these progenitors, such as GLAST$^+$ Wnt1$^+$ BM-SCs, circulate in low amounts through peripheral blood under physiological conditions and appear to contribute cells from different embryonic layers; this does not necessary mean they are pluripotent. Another study showed that circulating mouse CD11b$^-$ CD29$^+$ CD90$^+$ CD106$^+$ BM-SCs originate cells the corneal epithelium during the regeneration of this tissue (Hong et al., 2009). It remains to be elucidated whether or not this population overlaps with GLAST$^+$ Wnt1$^+$ BM-SCs. Cells that move into the bloodstream are likely to respond to a gradient of signals derived from damaged/stressed tissues and are recruited into them to facilitate healing and/or regeneration. It is intriguing how some cell types appear to maintain such increased developmental plasticity in adulthood and what is its significance, since their contribution to cells in distant tissues appears very limited. These mechanisms involve epigenetic processes, and could include cell fusion and/or microvesicles interchange, and hypoxic and/or stress conditions at niche microenvironment.

Cell fusion first results in heterokaryons. In some cases, heterokaryons can enter the cell cycle and, in that case, their two nuclei would fuse (synkaryon). Hybrids originate through this process, a feature first described using cell lines (Barski et al., 1960). The transition from a heterokaryon to a synkaryon was associated with aneuploidy/genomic instability during subsequent cell divisions (Dornen et al., 2020). Aneuploidy was also described in human MSCs already in passage 3 (Kim et al., 2015), with the occurrence of cellular senescence (Bernardo et al., 2007). It remains to be elucidated whether or not hybrids exist in MSCs cultures, and to what extent such phenomena could be relevant in the genomic instability of MSCs. Cell fusion has been involved in cell reprogramming and regenerative mechanisms (Lluis and Cosma, 2010; Acquistapace et al., 2011). By coculturing adult mouse cardiomyocytes with human adipose MSCs, Acquistapace et al. (2011) showed reprogramming of cardiomyocytes to a progenitor-like state, caused by their transient fusion with MSCs. In this case, cells were shown to exchange mitochondria and small organelles. Furthermore, heterokaryons were observed *ex vivo* when human MSCs were cocultured with airway epithelial cells, resulting in the maintenance of the epithelial phenotype (Spees et al., 2003). In this study, a significant fraction of the fused cells contained a single nucleus and evidence of cell hybrids was also described. *In vivo* fusion events of MSCs/BM-SCs with host tissue cells could be relevant in the context of tissue inflammation, an issue that for the moment has only been demonstrated *in vitro* (Dornen et al., 2020; Kemp et al., 2011). With regard specifically to ECs/EPCs, their fusion with other cell types, such as cardiomyocytes or hepatocytes, was described: it was shown that these events promote the proliferation of these latter cell types, both *in vitro* and *in vivo* (Matsuura et al., 2004; Pu et al., 2020). In addition, EPCs were observed to exchange membrane and organelles through transient 50–800 μm intercellular nanotubular processes with cardiomyocytes (Koyanagi et al., 2005).

Alternatively, cell reprogramming can take place through **microvesicles**, which contain mRNA and proteins (Zhou et al., 2016b),

released by cells. These events can be especially relevant *in vitro* and would be expected when incubating cells in culture medium supplemented with pro-proliferative growth factors and/or on surfaces coated with extracellular matrix proteins (Yan et al., 2020), as is the case of MAPCs and MIAMI cells. With regard to ECs, they were shown to express tetraspanin CD9 (Franz et al., 2016), a protein involved in cell fusion (Brukman et al., 2019), and in exosomes delivery, adhesion and fusion (Reyes et al., 2018; Boker et al., 2018). In a recent publication, Yan et al. (2020) reported that platelets, as well as microvesicles released by them, can induce proliferation of EPCs in a rat model of carotid artery intimal injury *in vivo*, through TGF-β1 signaling. In this publication, TGF-β1 levels within extracellular microvesicles were found to be increased when platelets were pre-incubated in collagen, and such a condition resulted in a significant improvement in the proliferation of EPCs. Therefore, cell fusion, microvesicles exchange and the formation of transient intercellular nanotube connections are known mechanisms that influence ECs, probably through epigenetic changes (Camussi et al., 2011). Furthermore, high concentrations of extracellular molecules within the bone marrow or under *in vitro* conditions could enhance the properties of increased plasticity, without resulting in cellular transdifferentiation (Bedada et al., 2006).

Among other environmental conditions, **hypoxia** is usually necessary for stem cells quiescence and characterizes their niches (Huang et al., 2018) (Fig. 1). Furthermore, hypoxia was shown to induce the expression of pluripotency markers in some adult stem cells (Werle et al., 2019; Sugimoto et al., 2018; Prasad et al., 2017; Heneidi et al., 2013). It is well known that glycolysis predominates in pluripotent stem cells (Nishimura et al., 2019). This metabolic feature is required for the self-renewal and pluripotency of stem cells and might explain their preference for regions with low oxygen tension. Moreover, a shift towards the acquisition of glycolytic metabolism was observed during somatic cell reprogramming into induced pluripotent stem cells (Nishimura et al., 2019). Interestingly, despite the large abundance of small vessels, much lower pO_2 (~10 mmHg) were reported in deep perisinusoidal areas of mouse bone marrow compared to compact bone endosteal and periarteriolar regions (~22–23 mmHg) (Spencer et al., 2014). In this study, $Nestin^+$ BM-SCs were found around large-diameter vessels and/or located closer to the endosteum (at <20 μm from it), in areas with relative higher levels of pO_2 (>20 mmHg) in comparison with those populated by of $Nestin^-$ stromal cells (<20 mmHg). It is worth mentioning that the $GLAST^+$ $Wnt1^+$ BM-SCs that contribute with ECs and HLCs are $Nestin^{-/low}$, and would be located in hypoxic bone marrow areas. Hypoxia has recently been shown to induce exosome production *in vivo* and *in vitro* (Zhang et al., 2017; Kim et al., 2018), a feature linked to epigenetic mechanisms (Choudhry and Harris, 2018).

Stress was also related to somatic and stem cells reprogramming (Kuroda et al., 2010; Parfejevs et al., 2018). Currently some intracellular mechanisms involved in the cellular response to stress are better known: they include the regulation of nucleolar function and translational reprogramming with the participation of long non-coding RNAs and epigenetic changes, to adapt to such conditions (Verheyden et al., 2018). Endoplasmic reticulum stress was associated to stemness and pluripotency (Kratochvilova et al., 2016), a feature that is also caused by hypoxia (Heneidi et al., 2013). It is well known that stem cells show greater tolerance to stress, especially after hypoxic preconditioning, and that they can survive better when placed in acutely injured areas (Kuroda et al., 2010; Heneidi et al., 2013).

8. Conclusions and future perspectives

Herein, evidences on different origins of ECs, mainly in adults, were discussed and are summarized in Fig. 1. The current literature suggests that, un adults, CEs of large vessels originate more frequently from vascular resident progenitors, of the intima and also of an area between the tunica media and adventitia, in the context of a physiological conditions/mild injuries versus severe injuries, respectively. Some recently discovered EC markers are helping to establish a developmental hierarchy between different subpopulations of EPCs in various tissues. Furthermore, circulating peripheral blood EPCs/ECs, $GLAST^+$ $Wnt1^+$ BM-SCs and/or possibly other progenitors, derived mainly from bone marrow, could contribute to a lesser extent with ECs *in vivo*.

The bone marrow is characterized by extensive areas of low pO_2, a condition that likely affects the expression profile and properties (including plasticity, proliferation and migratory capacities) of different stromal progenitor subpopulations. In this review, mouse and human BM-SCs capable of contributing to MSCs cultures were described, including different types of CAR cells, periarteriolar stromal cells and fibroblasts. Even though numerous scRNA-seq studies were conducted to characterize subpopulations of mouse BM-SCs, the heterogeneity between perisinusoidal CAR cells is greater than expected, so new single cell expression profile analyses but focused on this specific subpopulation are required. Likewise, it would be expected that the functional properties of these different subgroups differ greatly. In fact, during liver fibrogenesis and regeneration, $GLAST^+$ $Wnt1^+$ BM-SCs, a small subpopulation of perisinusoidal cells, could contribute to ELCs and HLCs. Under specific culture conditions, such as coating of surfaces with extracellular matrix proteins, the supplementation of the culture medium with mitogenic growth factors and/or hypoxia/stress, various cell types with some common characteristics with pluripotent cells were obtained from bone marrow mononuclear cell/MSCs cultures, including MAPCs, MIAMI and Muse cells. These, and perhaps other cell types as well, could also differentiate into ELCs, a topic that requires further investigation to exclude EPCs contamination from initial cultures. There are also reports of a differentiation into ELCs of cells lacking an EPC/EC phenotype from adipose, neural and dental pulp tissue, with possible implications in future tissue engineering/replacement strategies. In the bone marrow and bloodstream, $GLAST^+$ $Wnt1^+$ BM-SCs lacked EPC/EC markers. Therefore, these cells with the potential to originate ELCs could be considered functional angioblasts. It remains to be addressed whether or not $GLAST^+$ $Wnt1^+$ BM-SCs can originate, *in vitro,* MAPCs or other previously reported cells with increased plasticity. These *in vitro* conditions could affect differently the expression profile of diverse stromal progenitor subpopulations, through reprogramming; therefore, in that case some established markers for different pluripotent-like cells *in vitro* could be absent *in vivo* in the cells that originate them. Two main sources of adult circulating EPCs have traditionally been considered: early EPCs and late EPCs. The fact that early EPCs show an expression profile similar to that of myelomonocytes could argue against any differentiation of hematopoietic cells into EPCs; instead, they can result from the fusion of cells or microvesicles. Furthermore, the hypothesis of the existence of hemangioblasts remains controversial. On the other hand, late EPCs have a typical endothelial-like profile. It should be noted that, until recently, these bone marrow-derived late EPCs were considered the main source of ECs in adults; this hypothesis was rejected by new data from different independent groups. Bone marrow sinusoidal areas, where CAR cells are found, are characterized by a hypoxic microenvironment that would probably influence such stromal progenitors epigenetically. This influence could explain the high heterogeneity observed in CAR cells and could eventually result in their enhanced plasticity, as well as in their ability to proliferate, mobilize and recruit in injured tissues. Among other mechanisms, hypoxia could increase exchange of microvesicles between cells. It remains to be addressed whether $GLAST^+$ $Wnt1^+$ BM-SCs can have more extensive active genomic regions compared to other BM-SCs; indeed, elevated levels of specific markers characterizing different subpopulations of the bone marrow stroma were observed in groups of CAR cells (Baryawno et al., 2019). New tools are now available to analyze, in individual cells, their mRNA expression profiles (scRNA-seq) as well as their active genomic regions (such as scATAC-seq; an assay based on transposase-accessible chromatin sequencing) (Stuart et al., 2019). These combined approaches may be helpful to identify different bone marrow CAR stromal cells subpopulations and changes in their expression pattern and chromatin modifications throughout early postnatal development

and also after injury. Furthermore, they would help to discover mechanisms involved during acquisition of pluripotent-like properties by MSCs *in vitro*. In this sense, a new technique called LARRY (for lineage and RNA recovery) was recently published: it is based on a modification of the standard clonal labeling through lentiviral delivery of inherited DNA barcodes, which allows barcode detection using scRNA-seq (Weinreb et al., 2020). LARRY could help distinguish the lineage of very different progenitor cell types in parallel and over time. By all these means, we hope to be able to better understand in the next future the mechanisms involved in the origin of ECs/ELCs from such numerous and diverse sources in the adult and specifically in the context of different disease models in which they could be involved.

Acknowledgements:

This work has been funded by FONCyT-ANPCyT (PICT-O-2016-0088 and PICT-2017-0642) and Austral University. The authors have no conflicts of interest.

References

Abbott, A., 2013. Doubt cast over tiny stem cells. Nature 499 (7459), 390. https://doi.org/10.1038/499390a.

Acquistapace, A., Bru, T., Lesault, P.F., Figeac, F., Coudert, A.E., le Coz, O., Christov, C., Baudin, X., Auber, F., Yiou, R., Dubois-Rande, J.L., Rodriguez, A.M., 2011. Human mesenchymal stem cells reprogram adult cardiomyocytes toward a progenitor-like state through partial cell fusion and mitochondria transfer. Stem cells 29 (5), 812–824. https://doi.org/10.1002/stem.632.

Aksel, H., Huang, G.T., 2017. Human and swine dental pulp stem cells form a vascularlike network after angiogenic differentiation in comparison with endothelial cells: a quantitative analysis. J. Endod. 43 (4), 588–595. https://doi.org/10.1016/j.joen.2016.11.015.

Ambler, C.A., Nowicki, J.L., Burke, A.C., Bautch, V.L., 2001. Assembly of trunk and limb blood vessels involves extensive migration and vasculogenesis of somite-derived angioblasts. Dev. Biol. 234 (2), 352–364. https://doi.org/10.1006/dbio.2001.0267.

Asada, N., Kunisaki, Y., Pierce, H., Wang, Z., Fernandez, N.F., Birbrair, A., Ma'ayan, A., Frenette, P.S., 2017. Differential cytokine contributions of perivascular haematopoietic stem cell niches. Nat. Cell Biol. 19 (3), 214–223. https://doi.org/10.1038/ncb3475.

Asahara, T., Murohara, T., Sullivan, A., Silver, M., van der Zee, R., Li, T., Witzenbichler, B., Schatteman, G., Isner, J.M., 1997. Isolation of putative progenitor endothelial cells for angiogenesis. Science 275 (5302), 964–967. https://doi.org/10.1126/science.275.5302.964.

Asahara, T., Masuda, H., Takahashi, T., Kalka, C., Pastore, C., Silver, M., Kearne, M., Magner, M., Isner, J.M., 1999. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. Circ. Res. 85 (3), 221–228. https://doi.org/10.1161/01.res.85.3.221.

Assmus, B., Iwasaki, M., Schachinger, V., Roexe, T., Koyanagi, M., Iekushi, K., Xu, Q., Tonn, T., Seifried, E., Liebner, S., Kranert, W.T., Grunwald, F., Dimmeler, S., Zeiher, A.M., 2012. Acute myocardial infarction activates progenitor cells and increases Wnt signalling in the bone marrow. Eur. Heart J. 33 (15), 1911–1919. https://doi.org/10.1093/eurheartj/ehr388.

Astrof, S., Hynes, R.O., 2009. Fibronectins in vascular morphogenesis. Angiogenesis 12 (2), 165–175. https://doi.org/10.1007/s10456-009-9136-6.

Azevedo Portilho, N., Pelajo-Machado, M., 2018. Mechanism of hematopoiesis and vasculogenesis in mouse placenta. Placenta 69, 140–145. https://doi.org/10.1016/j.placenta.2018.04.007.

Baccin, C., Al-Sabah, J., Velten, L., Helbling, P.M., Grunschlager, F., Hernandez-Malmierca, P., Nombela-Arrieta, C., Steinmetz, L.M., Trumpp, A., Haas, S., 2020. Combined single-cell and spatial transcriptomics reveal the molecular, cellular and spatial bone marrow niche organization. Nat. Cell Biol. 22 (1), 38–48. https://doi.org/10.1038/s41556-019-0439-6.

Balakrishnan, A., Stykel, M.G., Touahri, Y., Stratton, J.A., Biernaskie, J., Schuurmans, C., 2016. Temporal analysis of gene expression in the murine schwann cell lineage and the acutely injured postnatal nerve. PloS One 11 (4), e0153256. https://doi.org/10.1371/journal.pone.0153256.

Bao, J., Wu, Q., Wang, Y., Li, Y., Li, L., Chen, F., Wu, X., Xie, M., Bu, H., 2016. Enhanced hepatic differentiation of rat bone marrow-derived mesenchymal stem cells in spheroidal aggregate culture on a decellularized liver scaffold. Int. J. Mol. Med. 38 (2), 457–465. https://doi.org/10.3892/ijmm.2016.2638.

Baron, C.S., Kester, L., Klaus, A., Boisset, J.C., Thambyrajah, R., Yvernogeau, L., Kouskoff, V., Lacaud, G., van Oudenaarden, A., Robin, C., 2018. Single-cell transcriptomics reveal the dynamic of haematopoietic stem cell production in the aorta. Nat. Commun. 9 (1), 2517. https://doi.org/10.1038/s41467-018-04893-3.

Barski, G., Sorieul, S., Cornefert, F., 1960. [Production of cells of a "hybrid" nature in culturs in vitro of 2 cellular strains in combination]. Comptes rendus hebdomadaires des seances de l'Academie des sciences 251, 1825–1827.

Baryawno, N., Przybylski, D., Kowalczyk, M.S., Kfoury, Y., Severe, N., Gustafsson, K., Kokkaliaris, K.D., Mercier, F., Tabaka, M., Hofree, M., Dionne, D., Papazian, A., Lee, D., Ashenberg, O., Subramanian, A., Vaishnav, E.D., Rozenblatt-Rosen, O., Regev, A., Scadden, D.T., 2019. A cellular taxonomy of the bone marrow stroma in homeostasis and leukemia. Cell 177 (7), 1915–1932. https://doi.org/10.1016/j.cell.2019.04.040 e1916.

Battula, V.L., Treml, S., Bareiss, P.M., Gieseke, F., Roelofs, H., de Zwart, P., Muller, I., Schewe, B., Skutella, T., Fibbe, W.E., Kanz, L., Buhring, H.J., 2009. Isolation of functionally distinct mesenchymal stem cell subsets using antibodies against CD56, CD271, and mesenchymal stem cell antigen-1. Haematologica 94 (2), 173–184. https://doi.org/10.3324/haematol.13740.

Bedada, F.B., Gunther, S., Kubin, T., Braun, T., 2006. Differentiation versus plasticity: fixing the fate of undetermined adult stem cells. Cell Cycle 5 (3), 223–226. https://doi.org/10.4161/cc.5.3.2364.

Bernardo, M.E., Zaffaroni, N., Novara, F., Cometa, A.M., Avanzini, M.A., Moretta, A., Montagna, D., Maccario, R., Villa, R., Daidone, M.G., Zuffardi, O., Locatelli, F., 2007. Human bone marrow derived mesenchymal stem cells do not undergo transformation after long-term in vitro culture and do not exhibit telomere maintenance mechanisms. Canc. Res. 67 (19), 9142–9149. https://doi.org/10.1158/0008-5472.CAN-06-4690.

Bertrand, J.Y., Chi, N.C., Santoso, B., Teng, S., Stainier, D.Y., Traver, D., 2010. Haematopoietic stem cells derive directly from aortic endothelium during development. Nature 464 (7285), 108–111. https://doi.org/10.1038/nature08738.

Blaue, C., Kashef, J., Franz, C.M., 2018. Cadherin-11 promotes neural crest cell spreading by reducing intracellular tension-Mapping adhesion and mechanics in neural crest explants by atomic force microscopy. Semin. Cell Dev. Biol. 73, 95–106. https://doi.org/10.1016/j.semcdb.2017.08.058.

Boiret, N., Rapatel, C., Veyrat-Masson, R., Guillouard, L., Guerin, J.J., Pigeon, P., Descamps, S., Boisgard, S., Berger, M.G., 2005. Characterization of nonexpanded mesenchymal progenitor cells from normal adult human bone marrow. Exp. Hematol. 33 (2), 219–225. https://doi.org/10.1016/j.exphem.2004.11.001.

Boisset, J.C., van Cappellen, W., Andrieu-Soler, C., Galjart, N., Dzierzak, E., Robin, C., 2010. In vivo imaging of haematopoietic cells emerging from the mouse aortic endothelium. Nature 464 (7285), 116–120. https://doi.org/10.1038/nature08764.

Boker, K.O., Lemus-Diaz, N., Rinaldi Ferreira, R., Schiller, L., Schneider, S., Gruber, J., 2018. The impact of the CD9 tetraspanin on lentivirus infectivity and exosome secretion. Mol. Ther. : the journal of the American Society of Gene Therapy 26 (2), 634–647. https://doi.org/10.1016/j.ymthe.2017.11.008.

Brukman, N.G., Uygur, B., Podbilewicz, B., Chernomordik, L.V., 2019. How cells fuse. J. Cell Biol. 218 (5), 1436–1451. https://doi.org/10.1083/jcb.201901017.

Camargo, F.D., Green, R., Capetanaki, Y., Jackson, K.A., Goodell, M.A., 2003. Single hematopoietic stem cells generate skeletal muscle through myeloid intermediates. Nat. Med. 9 (12), 1520–1527. https://doi.org/10.1038/nm963.

Camargo, F.D., Finegold, M., Goodell, M.A., 2004. Hematopoietic myelomonocytic cells are the major source of hepatocyte fusion partners. J. Clin. Invest. 113 (9), 1266–1270. https://doi.org/10.1172/JCI21301.

Camussi, G., Deregibus, M.C., Bruno, S., Grange, C., Fonsato, V., Tetta, C., 2011. Exosome/microvesicle-mediated epigenetic reprogramming of cells. American journal of cancer research 1 (1), 98–110.

Chen, M.J., Li, Y., De Obaldia, M.E., Yang, Q., Yzaguirre, A.D., Yamada-Inagawa, T., Vink, C.S., Bhandoola, A., Dzierzak, E., Speck, N.A., 2011. Erythroid/myeloid progenitors and hematopoietic stem cells originate from distinct populations of endothelial cells. Cell stem cell 9 (6), 541–552. https://doi.org/10.1016/j.stem.2011.10.003.

Choudhry, H., Harris, A.L., 2018. Advances in hypoxia-inducible factor biology. Cell Metabol. 27 (2), 281–298. https://doi.org/10.1016/j.cmet.2017.10.005.

Cines, D.B., Pollak, E.S., Buck, C.A., Loscalzo, J., Zimmerman, G.A., McEver, R.P., Pober, J.S., Wick, T.M., Konkle, B.A., Schwartz, B.S., Barnathan, E.S., McCrae, K.R., Hug, B.A., Schmidt, A.M., Stern, D.M., 1998. Endothelial cells in physiology and in the pathophysiology of vascular disorders. Blood 91 (10), 3527–3561.

Cordeiro, M.M., Dong, Z., Kaneko, T., Zhang, Z., Miyazawa, M., Shi, S., Smith, A.J., Nor, J.E., 2008. Dental pulp tissue engineering with stem cells from exfoliated deciduous teeth. J. Endod. 34 (8), 962–969. https://doi.org/10.1016/j.joen.2008.04.009.

d'Aquino, R., Graziano, A., Sampaolesi, M., Laino, G., Pirozzi, G., De Rosa, A., Papaccio, G., 2007. Human postnatal dental pulp cells co-differentiate into osteoblasts and endotheliocytes: a pivotal synergy leading to adult bone tissue formation. Cell Death Differ. 14 (6), 1162–1171. https://doi.org/10.1038/sj.cdd.4402121.

D'Ippolito, G., Diabira, S., Howard, G.A., Menei, P., Roos, B.A., Schiller, P.C., 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 (Pt 14), 2971–2981. https://doi.org/10.1242/jcs.01103.

Danova-Alt, R., Heider, A., Egger, D., Cross, M., Alt, R., 2012. Very small embryonic-like stem cells purified from umbilical cord blood lack stem cell characteristics. PloS One 7 (4), e34899. https://doi.org/10.1371/journal.pone.0034899.

de Bruijn, M.F., Speck, N.A., Peeters, M.C., Dzierzak, E., 2000. Definitive hematopoietic stem cells first develop within the major arterial regions of the mouse embryo. EMBO J. 19 (11), 2465–2474. https://doi.org/10.1093/emboj/19.11.2465.

Deng, J., Ni, Z., Gu, W., Chen, Q., Nowak, W.N., Chen, T., Issa Bhaloo, S., Zhang, Z., Hu, Y., Zhou, B., Zhang, L., Xu, Q., 2020. Single-cell gene profiling and lineage tracing analyses revealed novel mechanisms of endothelial repair by progenitors. Cell. Mol. Life Sci. : CM. https://doi.org/10.1007/s00018-020-03480-4.

Dornen, J., Sieler, M., Weiler, J., Keil, S., Dittmar, T., 2020. Cell fusion-mediated tissue regeneration as an inducer of polyploidy and aneuploidy. Int. J. Mol. Sci. 21 (5) https://doi.org/10.3390/ijms21051811.

Drake, C.J., Fleming, P.A., 2000. Vasculogenesis in the day 6.5 to 9.5 mouse embryo. Blood 95 (5), 1671–1679.

Dyer, M.A., Farrington, S.M., Mohn, D., Munday, J.R., Baron, M.H., 2001. Indian hedgehog activates hematopoiesis and vasculogenesis and can respecify prospective neurectodermal cell fate in the mouse embryo. Development 128 (10), 1717–1730.
Dzierzak, E., Bigas, A., 2018. Blood development: hematopoietic stem cell dependence and independence. Cell stem cell 22 (5), 639–651. https://doi.org/10.1016/j.stem.2018.04.015.
Fang, S., Wei, J., Pentinmikko, N., Leinonen, H., Salven, P., 2012. Generation of functional blood vessels from a single c-kit+ adult vascular endothelial stem cell. PLoS Biol. 10 (10), e1001407 https://doi.org/10.1371/journal.pbio.1001407.
Ferkowicz, M.J., Yoder, M.C., 2005. Blood island formation: longstanding observations and modern interpretations. Exp. Hematol. 33 (9), 1041–1047. https://doi.org/10.1016/j.exphem.2005.06.006.
Fiore, E.J., Mazzolini, G., Aquino, J.B., 2015. Mesenchymal stem/stromal cells in liver fibrosis: recent findings, old/new caveats and future perspectives. Stem cell reviews and reports 11 (4), 586–597. https://doi.org/10.1007/s12015-015-9585-9.
Franco, C.A., Jones, M.L., Bernabeu, M.O., Geudens, I., Mathivet, T., Rosa, A., Lopes, F.M., Lima, A.P., Ragab, A., Collins, R.T., Phng, L.K., Coveney, P.V., Gerhardt, H., 2015. Dynamic endothelial cell rearrangements drive developmental vessel regression. PLoS Biol. 13 (4), e1002125 https://doi.org/10.1371/journal.pbio.1002125.
Franz, J., Brinkmann, B.F., Konig, M., Huve, J., Stock, C., Ebnet, K., Riethmuller, C., 2016. Nanoscale imaging reveals a tetraspanin-CD9 coordinated elevation of endothelial ICAM-1 clusters. PloS One 11 (1), e0146598. https://doi.org/10.1371/journal.pone.0146598.
Fujisawa, T., Tura-Ceide, O., Hunter, A., Mitchell, A., Vesey, A., Medine, C., Gallogly, S., Hadoke, P.W.F., Keith, C., Sproul, A., Roddie, H., McQuaker, G., Wilmut, I., Mills, N.L., Brittan, M., 2019. Endothelial progenitor cells do not originate from the bone marrow. Circulation 140 (18), 1524–1526. https://doi.org/10.1161/CIRCULATIONAHA.119.042351.
George, F., Poncelet, P., Laurent, J.C., Massot, O., Arnoux, D., Lequeux, N., Ambrosi, P., Chicheportiche, C., Sampol, J., 1991. Cytofluorometric detection of human endothelial cells in whole blood using S-Endo 1 monoclonal antibody. J. Immunol. Methods 139 (1), 65–75. https://doi.org/10.1016/0022-1759(91)90352-g.
Gimeno, M.L., Fuertes, F., Barcala Tabarrozzi, A.E., Attorressi, A.I., Cucchiani, R., Corrales, L., Oliveira, T.C., Sogayar, M.C., Labriola, L., Dewey, R.A., Perone, M.J., 2017. Pluripotent nontumorigenic adipose tissue-derived Muse cells have immunomodulatory capacity mediated by transforming growth factor-beta1. Stem cells translational medicine 6 (1), 161–173. https://doi.org/10.5966/sctm.2016-0014.
Gomez Perdiguero, E., Klapproth, K., Schulz, C., Busch, K., Azzoni, E., Crozet, L., Garner, H., Trouillet, C., de Bruijn, M.F., Geissmann, F., Rodewald, H.R., 2015. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. Nature 518 (7540), 547–551. https://doi.org/10.1038/nature13989.
Grau-Monge, C., Delcroix, G.J., Bonnin-Marquez, A., Valdes, M., Awadallah, E.L., Quevedo, D.F., Armour, M.R., Montero, R.B., Schiller, P.C., Andreopoulos, F.M., D'Ippolito, G., 2017. Marrow-isolated adult multilineage inducible cells embedded within a biologically-inspired construct promote recovery in a mouse model of peripheral vascular disease. Biomed. Mater. 12 (1), 015024 https://doi.org/10.1088/1748-605X/aa5a74.
Gronthos, S., Mankani, M., Brahim, J., Robey, P.G., Shi, S., 2000. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc. Natl. Acad. Sci. U.S.A. 97 (25), 13625–13630. https://doi.org/10.1073/pnas.240309797.
Guerin, C.L., Loyer, X., Vilar, J., Cras, A., Mirault, T., Gaussem, P., Silvestre, J.S., Smadja, D.M., 2015. Bone-marrow-derived very small embryonic-like stem cells in patients with critical leg ischaemia: evidence of vasculogenic potential. Thromb. Haemostasis 113 (5), 1084–1094. https://doi.org/10.1160/TH14-09-0748.
Guerin, C.L., Rossi, E., Saubamea, B., Cras, A., Mignon, V., Silvestre, J.S., Smadja, D.M., 2017. Human very small embryonic-like cells support vascular maturation and therapeutic revascularization induced by endothelial progenitor cells. Stem cell reviews and reports 13 (4), 552–560. https://doi.org/10.1007/s12015-017-9731-7.
Haar, J.L., Ackerman, G.A., 1971. A phase and electron microscopic study of vasculogenesis and erythropoiesis in the yolk sac of the mouse. Anat. Rec. 170 (2), 199–223. https://doi.org/10.1002/ar.1091700206.
Heglind, M., Cederberg, A., Aquino, J., Lucas, G., Ernfors, P., Enerback, S., 2005. Lack of the central nervous system- and neural crest-expressed forkhead gene Foxs1 affects motor function and body weight. Mol. Cell Biol. 25 (13), 5616–5625. https://doi.org/10.1128/MCB.25.13.5616-5625.2005.
Hellwig, S.M., Damen, C.A., van Adrichem, N.P., Blijham, G.H., Groenewegen, G., Griffioen, A.W., 1997. Endothelial CD34 is suppressed in human malignancies: role of angiogenic factors. Canc. Lett. 120 (2), 203–211. https://doi.org/10.1016/s0304-3835(97)00310-8.
Heneidi, S., Simerman, A.A., Keller, E., Singh, P., Li, X., Dumesic, D.A., Chazenbalk, G., 2013. Awakened by cellular stress: isolation and characterization of a novel population of pluripotent stem cells derived from human adipose tissue. PloS One 8 (6), e64752. https://doi.org/10.1371/journal.pone.0064752.
Herrmann, M., Hildebrand, M., Menzel, U., Fahy, N., Alini, M., Lang, S., Benneker, L., Verrier, S., Stoddart, M.J., Bara, J.J., 2019. Phenotypic characterization of bone marrow mononuclear cells and derived stromal cell populations from human iliac crest, vertebral body and femoral head. Int. J. Mol. Sci. 20 (14) https://doi.org/10.3390/ijms20143454.
Hong, H.S., Lee, J., Lee, E., Kwon, Y.S., Lee, E., Ahn, W., Jiang, M.H., Kim, J.C., Son, Y., 2009. A new role of substance P as an injury-inducible messenger for mobilization of CD29(+) stromal-like cells. Nat. Med. 15 (4), 425–435. https://doi.org/10.1038/nm.1909.
Hosoyama, K., Wakao, S., Kushida, Y., Ogura, F., Maeda, K., Adachi, O., Kawamoto, S., Dezawa, M., Saiki, Y., 2018. Intravenously injected human multilineage-differentiating stress-enduring cells selectively engraft into mouse aortic aneurysms and attenuate dilatation by differentiating into multiple cell types. J. Thorac. Cardiovasc. Surg. 155 (6), 2301–2313. https://doi.org/10.1016/j.jtcvs.2018.01.098 e2304.
Huang, X., Trinh, T., Aljoufi, A., Broxmeyer, H.E., 2018. Hypoxia signaling pathway in stem cell regulation: good and evil. Current stem cell reports 4 (2), 149–157. https://doi.org/10.1007/s40778-018-0127-7.
Huber, T.L., Kouskoff, V., Fehling, H.J., Palis, J., Keller, G., 2004. Haemangioblast commitment is initiated in the primitive streak of the mouse embryo. Nature 432 (7017), 625–630. https://doi.org/10.1038/nature03122.
Hughes, S., Chang-Ling, T., 2000. Roles of endothelial cell migration and apoptosis in vascular remodeling during development of the central nervous system. Microcirculation 7 (5), 317–333.
Hur, J., Yoon, C.H., Kim, H.S., Choi, J.H., Kang, H.J., Hwang, K.K., Oh, B.H., Lee, M.M., Park, Y.B., 2004. Characterization of two types of endothelial progenitor cells and their different contributions to neovasculogenesis. Arterioscler. Thromb. Vasc. Biol. 24 (2), 288–293. https://doi.org/10.1161/01.ATV.0000114236.77009.06.
Ii, M., Nishimura, H., Sekiguchi, H., Kamei, N., Yokoyama, A., Horii, M., Asahara, T., 2009. Concurrent vasculogenesis and neurogenesis from adult neural stem cells. Circ. Res. 105 (9), 860–868. https://doi.org/10.1161/CIRCRESAHA.109.199299.
Ilas, D.C., Baboolal, T.G., Churchman, S.M., Jones, W.G., Giannoudis, P.V., Buhring, H.J., McGonagle, D., Jones, E., 2020. The osteogenic commitment of CD271+CD56+ bone marrow stromal cells (BMSCs) in osteoarthritic femoral head bone. Sci. Rep. 10 (1), 11145. https://doi.org/10.1038/s41598-020-67998-0.
Iohara, K., Zheng, L., Wake, H., Ito, M., Nabekura, J., Wakita, H., Nakamura, H., Into, T., Matsushita, K., Nakashima, M., 2008. A novel stem cell source for vasculogenesis in ischemia: subfraction of side population cells from dental pulp. Stem cells 26 (9), 2408–2418. https://doi.org/10.1634/stemcells.2008-0393.
Ivanovs, A., Rybtsov, S., Welch, L., Anderson, R.A., Turner, M.L., Medvinsky, A., 2011. Highly potent human hematopoietic stem cells first emerge in the intraembryonic aorta-gonad-mesonephros region. J. Exp. Med. 208 (12), 2417–2427. https://doi.org/10.1084/jem.20111688.
Jiang, Y., Jahagirdar, B.N., Reinhardt, R.L., Schwartz, R.E., Keene, C.D., Ortiz-Gonzalez, X.R., Reyes, M., Lenvik, T., Lund, T., Blackstad, M., Du, J., Aldrich, S., Lisberg, A., Low, W.C., Largaespada, D.A., Verfaillie, C.M., 2002. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 418 (6893), 41–49. https://doi.org/10.1038/nature00870.
Jones, E.A., Kinsey, S.E., English, A., Jones, R.A., Straszynski, L., Meredith, D.M., Markham, A.F., Jack, A., Emery, P., McGonagle, D., 2002. Isolation and characterization of bone marrow multipotential mesenchymal progenitor cells. Arthritis Rheum. 46 (12), 3349–3360. https://doi.org/10.1002/art.10696.
Kee, Y., Bronner-Fraser, M., 2001. Id4 expression and its relationship to other Id genes during avian embryonic development. Mech. Dev. 109 (2), 341–345. https://doi.org/10.1016/s0925-4773(01)00576-7.
Kemp, K., Gordon, D., Wraith, D.C., Mallam, E., Hartfield, E., Uney, J., Wilkins, A., Scolding, N., 2011. Fusion between human mesenchymal stem cells and rodent cerebellar Purkinje cells. Neuropathol. Appl. Neurobiol. 37 (2), 166–178. https://doi.org/10.1111/j.1365-2990.2010.01122.x.
Kim, S.Y., Im, K., Park, S.N., Kwon, J., Kim, J.A., Choi, Q., Hwang, S.M., Han, S.H., Kwon, S., Oh, I.H., Lee, D.S., 2015. Asymmetric aneuploidy in mesenchymal stromal cells detected by in situ karyotyping and fluorescence in situ hybridization: suggestions for reference values for stem cells. Stem Cell. Dev. 24 (1), 77–92. https://doi.org/10.1089/scd.2014.0137.
Kim, M., Yun, H.W., Park, D.Y., Choi, B.H., Min, B.H., 2018. Three-dimensional spheroid culture increases exosome secretion from mesenchymal stem cells. Tissue engineering and regenerative medicine 15 (4), 427–436. https://doi.org/10.1007/s13770-018-0139-5.
Koyanagi, M., Brandes, R.P., Haendeler, J., Zeiher, A.M., Dimmeler, S., 2005. Cell-to-cell connection of endothelial progenitor cells with cardiac myocytes by nanotubes: a novel mechanism for cell fate changes? Circ. Res. 96 (10), 1039–1041. https://doi.org/10.1161/01.RES.0000168650.23479.0c.
Kratochvilova, K., Moran, L., Padourova, S., Stejskal, S., Tesarova, L., Simara, P., Hampl, A., Koutna, I., Vanhara, P., 2016. The role of the endoplasmic reticulum stress in stemness, pluripotency and development. Eur. J. Cell Biol. 95 (3–5), 115–123. https://doi.org/10.1016/j.ejcb.2016.02.002.
Kucia, M., Reca, R., Campbell, F.R., Zuba-Surma, E., Majka, M., Ratajczak, J., Ratajczak, M.Z., 2006. A population of very small embryonic-like (VSEL) CXCR4(+) SSEA-1(+)Oct-4+ stem cells identified in adult bone marrow. Leukemia 20 (5), 857–869. https://doi.org/10.1038/sj.leu.2404171.
Kucia, M.J., Wysoczynski, M., Wu, W., Zuba-Surma, E.K., Ratajczak, J., Ratajczak, M.Z., 2008. Evidence that very small embryonic-like stem cells are mobilized into peripheral blood. Stem cells 26 (8), 2083–2092. https://doi.org/10.1634/stemcells.2007-0922.
Kunisaki, Y., Bruns, I., Scheiermann, C., Ahmed, J., Pinho, S., Zhang, D., Mizoguchi, T., Wei, Q., Lucas, D., Ito, K., Mar, J.C., Bergman, A., Frenette, P.S., 2013. Arteriolar niches maintain haematopoietic stem cell quiescence. Nature 502 (7473), 637–643. https://doi.org/10.1038/nature12612.
Kuroda, Y., Kitada, M., Wakao, S., Nishikawa, K., Tanimura, Y., Makinoshima, H., Goda, M., Akashi, H., Inutsuka, A., Niwa, A., Shigemoto, T., Nabeshima, Y., Nakahata, T., Nabeshima, Y., Fujiyoshi, Y., Dezawa, M., 2010. Unique multipotent cells in adult human mesenchymal cell populations. In: Proceedings of the National Academy of Sciences of the United States of America, vol. 107, pp. 8639–8643. https://doi.org/10.1073/pnas.0911647107, 19.
Kusumbe, A.P., Ramasamy, S.K., Adams, R.H., 2014. Coupling of angiogenesis and osteogenesis by a specific vessel subtype in bone. Nature 507 (7492), 323–328. https://doi.org/10.1038/nature13145.

Lacaud, G., Kouskoff, V., 2017. Hemangioblast, hemogenic endothelium, and primitive versus definitive hematopoiesis. Exp. Hematol. 49, 19–24. https://doi.org/10.1016/j.exphem.2016.12.009.
Lawson, K.A., Meneses, J.J., Pedersen, R.A., 1991. Clonal analysis of epiblast fate during germ layer formation in the mouse embryo. Development 113 (3), 891–911.
Lenard, A., Daetwyler, S., Betz, C., Ellertsdottir, E., Belting, H.G., Huisken, J., Affolter, M., 2015. Endothelial cell self-fusion during vascular pruning. PLoS Biol. 13 (4), e1002126 https://doi.org/10.1371/journal.pbio.1002126.
Li, H., Zhu, L., Chen, H., Li, T., Han, Q., Wang, S., Yao, X., Feng, H., Fan, L., Gao, S., Boyd, R., Cao, X., Zhu, P., Li, J., Keating, A., Su, X., Zhao, R.C., 2018. Generation of functional hepatocytes from human adipose-derived MYC(+) KLF4(+) GMNN(+) stem cells analyzed by single-cell RNA-seq profiling. Stem cells translational medicine 7 (11), 792–805. https://doi.org/10.1002/sctm.17-0273.
Li, J., Perfetto, M., Materna, C., Li, R., Thi Tran, H., Vleminckx, K., Duncan, M.K., Wei, S., 2019. A new transgenic reporter line reveals Wnt-dependent Snai2 re-expression and cranial neural crest differentiation in Xenopus. Sci. Rep. 9 (1), 11191. https://doi.org/10.1038/s41598-019-47665-9.
Lin, Y., Weisdorf, D.J., Solovey, A., Hebbel, R.P., 2000. Origins of circulating endothelial cells and endothelial outgrowth from blood. J. Clin. Invest. 105 (1), 71–77. https://doi.org/10.1172/JCI8071.
Lluis, F., Cosma, M.P., 2010. Cell-fusion-mediated somatic-cell reprogramming: a mechanism for tissue regeneration. J. Cell. Physiol. 223 (1), 6–13. https://doi.org/10.1002/jcp.22003.
Luria, E.A., Panasyuk, A.F., Friedenstein, A.Y., 1971. Fibroblast colony formation from monolayer cultures of blood cells. Transfusion 11 (6), 345–349. https://doi.org/10.1111/j.1537-2995.1971.tb04426.x.
Mabuchi, Y., Morikawa, S., Harada, S., Niibe, K., Suzuki, S., Renault-Mihara, F., Houlihan, D.D., Akazawa, C., Okano, H., Matsuzaki, Y., 2013. LNGFR(+)THY-1(+) VCAM-1(hi+) cells reveal functionally distinct subpopulations in mesenchymal stem cells. Stem cell reports 1 (2), 152–165. https://doi.org/10.1016/j.stemcr.2013.06.001.
Madarampalli, B., Watts, G.F.M., Panipinto, P.M., Nguygen, H.N., Brenner, M.B., Noss, E.H., 2019. Interactions between cadherin-11 and platelet-derived growth factor receptor-alpha signaling link cell adhesion and proliferation. Biochim. Biophys. Acta (BBA) - Mol. Basis Dis. 1865 (6), 1516–1524. https://doi.org/10.1016/j.bbadis.2019.03.001.
Mahapatra, S., Firpo, M.T., Bacanamwo, M., 2010. Inhibition of DNA methyltransferases and histone deacetylases induces bone marrow-derived multipotent adult progenitor cells to differentiate into endothelial cells. Ethnicity & disease 20 (1 Suppl. 1). S1-60-64.
Marcelo, K.L., Goldie, L.C., Hirschi, K.K., 2013. Regulation of endothelial cell differentiation and specification. Circ. Res. 112 (9), 1272–1287. https://doi.org/10.1161/CIRCRESAHA.113.300506.
Marchionni, C., Bonsi, L., Alviano, F., Lanzoni, G., Di Tullio, A., Costa, R., Montanari, M., Tazzari, P.L., Ricci, F., Pasquinelli, G., Orrico, C., Grossi, A., Prati, C., Bagnara, G.P., 2009. Angiogenic potential of human dental pulp stromal (stem) cells. Int. J. Immunopathol. Pharmacol. 22 (3), 699–706. https://doi.org/10.1177/039463200902200315.
Marmigere, F., Montelius, A., Wegner, M., Groner, Y., Reichardt, L.F., Ernfors, P., 2006. The Runx1/AML1 transcription factor selectively regulates development and survival of TrkA nociceptive sensory neurons. Nat. Neurosci. 9 (2), 180–187. https://doi.org/10.1038/nn1631.
Matsuoka, Y., Nakatsuka, R., Sumide, K., Kawamura, H., Takahashi, M., Fujioka, T., Uemura, Y., Asano, H., Sasaki, Y., Inoue, M., Ogawa, H., Takahashi, T., Hino, M., Sonoda, Y., 2015. Prospectively isolated human bone marrow cell-derived MSCs support primitive human CD34-negative hematopoietic stem cells. Stem cells 33 (5), 1554–1565. https://doi.org/10.1002/stem.1941.
Matsuura, K., Wada, H., Nagai, T., Iijima, Y., Minamino, T., Sano, M., Akazawa, H., Molkentin, J.D., Kasanuki, H., Komuro, I., 2004. Cardiomyocytes fuse with surrounding noncardiomyocytes and reenter the cell cycle. J. Cell Biol. 167 (2), 351–363. https://doi.org/10.1083/jcb.200312111.
McGrath, K.E., Frame, J.M., Fegan, K.H., Bowen, J.R., Conway, S.J., Catherman, S.C., Kingsley, P.D., Koniski, A.D., Palis, J., 2015. Distinct sources of hematopoietic progenitors emerge before HSCs and provide functional blood cells in the mammalian embryo. Cell Rep. 11 (12), 1892–1904. https://doi.org/10.1016/j.celrep.2015.05.036.
Medina, R.J., O'Neill, C.L., Sweeney, M., Guduric-Fuchs, J., Gardiner, T.A., Simpson, D.A., Stitt, A.W., 2010. Molecular analysis of endothelial progenitor cell (EPC) subtypes reveals two distinct cell populations with different identities. BMC Med. Genom. 3, 18. https://doi.org/10.1186/1755-8794-3-18.
Medvinsky, A., Dzierzak, E., 1996. Definitive hematopoiesis is autonomously initiated by the AGM region. Cell 86 (6), 897–906. https://doi.org/10.1016/s0092-8674(00)80165-8.
Miranville, A., Heeschen, C., Sengenes, C., Curat, C.A., Busse, R., Bouloumie, A., 2004. Improvement of postnatal neovascularization by human adipose tissue-derived stem cells. Circulation 110 (3), 349–355. https://doi.org/10.1161/01.CIR.0000135466.16823.D0.
Miyanishi, M., Mori, Y., Seita, J., Chen, J.Y., Karten, S., Chan, C.K., Nakauchi, H., Weissman, I.L., 2013. Do pluripotent stem cells exist in adult mice as very small embryonic stem cells? Stem cell reports 1 (2), 198–208. https://doi.org/10.1016/j.stemcr.2013.07.001.
Muniz, C., Teodosio, C., Mayado, A., Amaral, A.T., Matarraz, S., Barcena, P., Sanchez, M.L., Alvarez-Twose, I., Diez-Campelo, M., Garcia-Montero, A.C., Blanco, J.F., Del Canizo, M.C., del Pino Montes, J., Orfao, A., 2015. Ex vivo identification and characterization of a population of CD13(high) CD105(+) CD45(-) mesenchymal stem cells in human bone marrow. Stem Cell Res. Ther. 6, 169. https://doi.org/10.1186/s13287-015-0152-8.
Naito, H., Wakabayashi, T., Kidoya, H., Muramatsu, F., Takara, K., Eino, D., Yamane, K., Iba, T., Takakura, N., 2016. Endothelial side population cells contribute to tumor angiogenesis and antiangiogenic drug resistance. Canc. Res. 76 (11), 3200–3210. https://doi.org/10.1158/0008-5472.CAN-15-2998.
Navarro, A., Marin, S., Riol, N., Carbonell-Uberos, F., Minana, M.D., 2015. Fibroblast-negative CD34-negative cells from human adipose tissue contain mesodermal precursors for endothelial and mesenchymal cells. Stem Cell. Dev. 24 (19), 2280–2296. https://doi.org/10.1089/scd.2015.0013.
Nicholls, H., 2013. VSELs: is ideology overtaking science? Cell stem cell 13 (2), 143–144. https://doi.org/10.1016/j.stem.2013.07.003.
Nishimura, K., Fukuda, A., Hisatake, K., 2019. Mechanisms of the metabolic shift during somatic cell reprogramming. Int. J. Mol. Sci. 20 (9) https://doi.org/10.3390/ijms20092254.
Oberlin, E., Tavian, M., Blazsek, I., Peault, B., 2002. Blood-forming potential of vascular endothelium in the human embryo. Development 129 (17), 4147–4157.
Oishi, K., Kobayashi, A., Fujii, K., Kanehira, D., Ito, Y., Uchida, M.K., 2004. Angiogenesis in vitro: vascular tube formation from the differentiation of neural stem cells. J. Pharmacol. Sci. 96 (2), 208–218. https://doi.org/10.1254/jphs.fp0040373.
Palis, J., McGrath, K.E., Kingsley, P.D., 1995. Initiation of hematopoiesis and vasculogenesis in murine yolk sac explants. Blood 86 (1), 156–163.
Palis, J., Robertson, S., Kennedy, M., Wall, C., Keller, G., 1999. Development of erythroid and myeloid progenitors in the yolk sac and embryo proper of the mouse. Development 126 (22), 5073–5084.
Pardanaud, L., Luton, D., Prigent, M., Bourcheix, L.M., Catala, M., Dieterlen-Lievre, F., 1996. Two distinct endothelial lineages in ontogeny, one of them related to hemopoiesis. Development 122 (5), 1363–1371.
Parfejevs, V., Antunes, A.T., Sommer, L., 2018. Injury and stress responses of adult neural crest-derived cells. Dev. Biol. 444 (Suppl. 1), S356–S365. https://doi.org/10.1016/j.ydbio.2018.05.011.
Pinho, S., Lacombe, J., Hanoun, M., Mizoguchi, T., Bruns, I., Kunisaki, Y., Frenette, P.S., 2013. PDGFRalpha and CD51 mark human nestin+ sphere-forming mesenchymal stem cells capable of hematopoietic progenitor cell expansion. J. Exp. Med. 210 (7), 1351–1367. https://doi.org/10.1084/jem.20122252.
Piryaei, A., Valojerdi, M.R., Shahsavani, M., Baharvand, H., 2011. Differentiation of bone marrow-derived mesenchymal stem cells into hepatocyte-like cells on nanofibers and their transplantation into a carbon tetrachloride-induced liver fibrosis model. Stem cell reviews and reports 7 (1), 103–118. https://doi.org/10.1007/s12015-010-9126-5.
Planat-Benard, V., Silvestre, J.S., Cousin, B., Andre, M., Nibbelink, M., Tamarat, R., Clergue, M., Manneville, C., Saillan-Barreau, C., Duriez, M., Tedgui, A., Levy, B., Penicaud, L., Casteilla, L., 2004. Plasticity of human adipose lineage cells toward endothelial cells: physiological and therapeutic perspectives. Circulation 109 (5), 656–663. https://doi.org/10.1161/01.CIR.0000114522.38265.61.
Prasad, P., Mittal, S.A., Chongtham, J., Mohanty, S., Srivastava, T., 2017. Hypoxia-mediated epigenetic regulation of stemness in brain tumor cells. Stem cells 35 (6), 1468–1478. https://doi.org/10.1002/stem.2621.
Prokopi, M., Pula, G., Mayr, U., Devue, C., Gallagher, J., Xiao, Q., Boulanger, C.M., Westwood, N., Urbich, C., Willeit, J., Steiner, M., Breuss, J., Xu, Q., Kiechl, S., Mayr, M., 2009. Proteomic analysis reveals presence of platelet microparticles in endothelial progenitor cell cultures. Blood 114 (3), 723–732. https://doi.org/10.1182/blood-2009-02-205930.
Pu, W., Han, X., Zhang, M., Li, Y., Huang, X., He, L., Zhou, B., 2020. Resident endothelial cells generate hepatocytes through cell fusion in adult mouse liver. Journal of genetics and genomics = Yi chuan xue bao 47 (4), 225–228. https://doi.org/10.1016/j.jgg.2020.03.006.
Pudliszewski, M., Pardanaud, L., 2005. Vasculogenesis and angiogenesis in the mouse embryo studied using quail/mouse chimeras. Int. J. Dev. Biol. 49 (2–3), 355–361. https://doi.org/10.1387/ijdb.041956mp.
Quirici, N., Soligo, D., Bossolasco, P., Servida, F., Lumini, C., Deliliers, G.L., 2002. Isolation of bone marrow mesenchymal stem cells by anti-nerve growth factor receptor antibodies. Exp. Hematol. 30 (7), 783–791. https://doi.org/10.1016/s0301-472x(02)00812-3.
Raemer, P.C., Haemmerling, S., Giese, T., Canaday, D.H., Katus, H.A., Dengler, T.J., Sivanandam, V.G., 2009. Endothelial progenitor cells possess monocyte-like antigen-presenting and T-cell-co-stimulatory capacity. Transplantation 87 (3), 340–349. https://doi.org/10.1097/TP.0b013e3181957308.
Rahnemai-Azar, A., D'Ippolito, G., Gomez, L.A., Reiner, T., Vazquez-Padron, R.I., Perez-Stable, C., Roos, B.A., Pham, S.M., Schiller, P.C., 2011. Human marrow-isolated adult multilineage-inducible (MIAMI) cells protect against peripheral vascular ischemia in a mouse model. Cytotherapy 13 (2), 179–192. https://doi.org/10.3109/14653249.2010.515579.
Ratajczak, M.Z., Mierzejewska, K., Ratajczak, J., Kucia, M., 2013. CD133 expression strongly correlates with the phenotype of very small embryonic-/epiblast-like stem cells. Adv. Exp. Med. Biol. 777, 125–141. https://doi.org/10.1007/978-1-4614-5894-4_9.
Ratajczak, M.Z., Zuba-Surma, E., Wojakowski, W., Suszynska, M., Mierzejewska, K., Liu, R., Ratajczak, J., Shin, D.M., Kucia, M., 2014. Very small embryonic-like stem cells (VSELs) represent a real challenge in stem cell biology: recent pros and cons in the midst of a lively debate. Leukemia 28 (3), 473–484. https://doi.org/10.1038/leu.2013.255.
Ratajczak, M.Z., Ratajczak, J., Kucia, M., 2019. Very small embryonic-like stem cells (VSELs). Circ. Res. 124 (2), 208–210. https://doi.org/10.1161/CIRCRESAHA.118.314287.

Reyes, M., Dudek, A., Jahagirdar, B., Koodie, L., Marker, P.H., Verfaillie, C.M., 2002. Origin of endothelial progenitors in human postnatal bone marrow. J. Clin. Invest. 109 (3), 337–346. https://doi.org/10.1172/JCI14327.

Reyes, R., Cardenes, B., Machado-Pineda, Y., Cabanas, C., 2018. Tetraspanin CD9: a key regulator of cell adhesion in the immune system. Front. Immunol. 9, 863. https://doi.org/10.3389/fimmu.2018.00863.

Rinkevich, Y., Lindau, P., Ueno, H., Longaker, M.T., Weissman, I.L., 2011. Germ-layer and lineage-restricted stem/progenitors regenerate the mouse digit tip. Nature 476 (7361), 409–413. https://doi.org/10.1038/nature10346.

Risau, W., Flamme, I., 1995. Vasculogenesis. Annu. Rev. Cell Dev. Biol. 11, 73–91. https://doi.org/10.1146/annurev.cb.11.110195.000445.

Ryu, J.C., Davidson, B.P., Xie, A., Qi, Y., Zha, D., Belcik, J.T., Caplan, E.S., Woda, J.M., Hedrick, C.C., Hanna, R.N., Lehman, N., Zhao, Y., Ting, A., Lindner, J.R., 2013. Molecular imaging of the paracrine proangiogenic effects of progenitor cell therapy in limb ischemia. Circulation 127 (6), 710–719. https://doi.org/10.1161/CIRCULATIONAHA.112.116103.

Sacchetti, B., Funari, A., Michienzi, S., Di Cesare, S., Piersanti, S., Saggio, I., Tagliafico, E., Ferrari, S., Robey, P.G., Riminucci, M., Bianco, P., 2007. Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. Cell 131 (2), 324–336. https://doi.org/10.1016/j.cell.2007.08.025.

Sakai, V.T., Zhang, Z., Dong, Z., Neiva, K.G., Machado, M.A., Shi, S., Santos, C.F., Nor, J.E., 2010. SHED differentiate into functional odontoblasts and endothelium. J. Dent. Res. 89 (8), 791–796. https://doi.org/10.1177/0022034510368647.

Sasaki, J.I., Zhang, Z., Oh, M., Pobocik, A.M., Imazato, S., Shi, S., Nor, J.E., 2020. VE-cadherin and anastomosis of blood vessels formed by dental stem cells. J. Dent. Res. 99 (4), 437–445. https://doi.org/10.1177/0022034520902458.

Sbarbati, R., de Boer, M., Marzilli, M., Scarlattini, M., Rossi, G., van Mourik, J.A., 1991. Immunologic detection of endothelial cells in human whole blood. Blood 77 (4), 764–769.

Shirai, Y., Kawabe, K., Tosa, I., Tsukamoto, S., Yamada, D., Takarada, T., 2019. Runx2 function in cells of neural crest origin during intramembranous ossification. Biochem. Biophys. Res. Commun. 509 (4), 1028–1033. https://doi.org/10.1016/j.bbrc.2019.01.059.

Sierra, R., Gomez Bustillo, S., Kameneva, P., Fiore, E.J., Mazzone, G.L., Borda, M., Blanco, M.V., Usuardi, C., Furlan, A., Ernfors, P., Alaniz, L., Montaner, A.D., Adameyko, I., Aquino, J.B., 2020. Contribution of neural crest and GLAST(+) Wnt1(+) bone marrow pericytes with liver fibrogenesis and/or regeneration. Liver Int. : official journal of the International Association for the Study of the Liver. https://doi.org/10.1111/liv.14401.

Soldatov, R., Kaucka, M., Kastriti, M.E., Petersen, J., Chontorotzea, T., Englmaier, L., Akkuratova, N., Yang, Y., Haring, M., Dyachuk, V., Bock, C., Farlik, M., Piacentino, M.L., Boismoreau, F., Hilscher, M.M., Yokota, C., Qian, X., Nilsson, M., Bronner, M.E., Croci, L., Hsiao, W.Y., Guertin, D.A., Brunet, J.F., Consalez, G.G., Ernfors, P., Fried, K., Kharchenko, P.V., Adameyko, I., 2019. Spatiotemporal structure of cell fate decisions in murine neural crest. Science 364 (6444). https://doi.org/10.1126/science.aas9536.

Spees, J.L., Olson, S.D., Ylostalo, J., Lynch, P.J., Smith, J., Perry, A., Peister, A., Wang, M.Y., Prockop, D.J., 2003. Differentiation, cell fusion, and nuclear fusion during ex vivo repair of epithelium by human adult stem cells from bone marrow stroma. Proc. Natl. Acad. Sci. U.S.A. 100 (5), 2397–2402. https://doi.org/10.1073/pnas.0437997100.

Spencer, J.A., Ferraro, F., Roussakis, E., Klein, A., Wu, J., Runnels, J.M., Zaher, W., Mortensen, L.J., Alt, C., Turcotte, R., Yusuf, R., Cote, D., Vinogradov, S.A., Scadden, D.T., Lin, C.P., 2014. Direct measurement of local oxygen concentration in the bone marrow of live animals. Nature 508 (7495), 269–273. https://doi.org/10.1038/nature13034.

Stuart, T., Butler, A., Hoffman, P., Hafemeister, C., Papalexi, E., Mauck 3rd, W.M., Hao, Y., Stoeckius, M., Smibert, P., Satija, R., 2019. Comprehensive integration of single-cell data. Cell 177 (7), 1888–1902. https://doi.org/10.1016/j.cell.2019.05.031 e1821.

Sugimoto, K., Matsuura, T., Nakazono, A., Igawa, K., Yamada, S., Hayashi, Y., 2018. Effects of hypoxia inducible factors on pluripotency in human iPS cells. Microsc. Res. Tech. 81 (7), 749–754. https://doi.org/10.1002/jemt.23032.

Sun, D., Yang, L., Cao, H., Shen, Z.Y., Song, H.L., 2020. Study of the protective effect on damaged intestinal epithelial cells of rat multilineage-differentiating stress-enduring (Muse) cells. Cell Biol. Int. 44 (2), 549–559. https://doi.org/10.1002/cbin.11255.

Suszynska, M., Zuba-Surma, E.K., Maj, M., Mierzejewska, K., Ratajczak, J., Kucia, M., Ratajczak, M.Z., 2014. The proper criteria for identification and sorting of very small embryonic-like stem cells, and some nomenclature issues. Stem Cell. Dev. 23 (7), 702–713. https://doi.org/10.1089/scd.2013.0472.

Szade, K., Bukowska-Strakova, K., Nowak, W.N., Szade, A., Kachamakova-Trojanowska, N., Zukowska, M., Jozkowicz, A., Dulak, J., 2013. Murine bone marrow Lin(-)Sca(-)1(+)CD45(-) very small embryonic-like (VSEL) cells are heterogeneous population lacking Oct-4A expression. PloS One 8 (5), e63329. https://doi.org/10.1371/journal.pone.0063329.

Tanaka, T., Nishigaki, K., Minatoguchi, S., Nawa, T., Yamada, Y., Kanamori, H., Mikami, A., Ushikoshi, H., Kawasaki, M., Dezawa, M., Minatoguchi, S., 2018. Mobilized Muse cells after acute myocardial infarction predict cardiac function and remodeling in the chronic phase. Circ. J. : official journal of the Japanese Circulation Society 82 (2), 561–571. https://doi.org/10.1253/circj.CJ-17-0552.

Taoudi, S., Medvinsky, A., 2007. Functional identification of the hematopoietic stem cell niche in the ventral domain of the embryonic dorsal aorta. Proc. Natl. Acad. Sci. U.S.A. 104 (22), 9399–9403. https://doi.org/10.1073/pnas.0700984104.

Tikhonova, A.N., Dolgalev, I., Hu, H., Sivaraj, K.K., Hoxha, E., Cuesta-Dominguez, A., Pinho, S., Akhmetzyanova, I., Gao, J., Witkowski, M., Guillamot, M., Gutkin, M.C., Zhang, Y., Marier, C., Diefenbach, C., Kousteni, S., Heguy, A., Zhong, H., Fooksman, D.R., Butler, J.M., Economides, A., Frenette, P.S., Adams, R.H., Satija, R., Tsirigos, A., Aifantis, I., 2019. The bone marrow microenvironment at single-cell resolution. Nature 569 (7755), 222–228. https://doi.org/10.1038/s41586-019-1104-8.

Tormin, A., Li, O., Brune, J.C., Walsh, S., Schutz, B., Ehinger, M., Ditzel, N., Kassem, M., Scheding, S., 2011. CD146 expression on primary nonhematopoietic bone marrow stem cells is correlated with in situ localization. Blood 117 (19), 5067–5077. https://doi.org/10.1182/blood-2010-08-304287.

Tura, O., Skinner, E.M., Barclay, G.R., Samuel, K., Gallagher, R.C., Brittan, M., Hadoke, P.W., Newby, D.E., Turner, M.L., Mills, N.L., 2013. Late outgrowth endothelial cells resemble mature endothelial cells and are not derived from bone marrow. Stem cells 31 (2), 338–348. https://doi.org/10.1002/stem.1280.

Uchida, N., Kushida, Y., Kitada, M., Wakao, S., Kumagai, N., Kuroda, Y., Kondo, Y., Hirohara, Y., Kure, S., Chazenbalk, G., Dezawa, M., 2017. Beneficial effects of systemically administered human Muse cells in adriamycin nephropathy. J. Am. Soc. Nephrol. : JASN (J. Am. Soc. Nephrol.) 28 (10), 2946–2960. https://doi.org/10.1681/ASN.2016070775.

Ueno, H., Weissman, I.L., 2006. Clonal analysis of mouse development reveals a polyclonal origin for yolk sac blood islands. Dev. Cell 11 (4), 519–533. https://doi.org/10.1016/j.devcel.2006.08.001.

Ulloa-Montoya, F., Kidder, B.L., Pauwelyn, K.A., Chase, L.G., Luttun, A., Crabbe, A., Geraerts, M., Sharov, A.A., Piao, Y., Ko, M.S., Hu, W.S., Verfaillie, C.M., 2007. Comparative transcriptome analysis of embryonic and adult stem cells with extended and limited differentiation capacity. Genome Biol. 8 (8), R163. https://doi.org/10.1186/gb-2007-8-8-r163.

Verheyden, Y., Goedert, L., Leucci, E., 2018. Control of nucleolar stress and translational reprogramming by lncRNAs. Cell stress 3 (1), 19–26. https://doi.org/10.15698/cst2019.01.172.

Wakabayashi, T., Naito, H., Suehiro, J.I., Lin, Y., Kawaji, H., Iba, T., Kouno, T., Ishikawa-Kato, S., Furuno, M., Takara, K., Muramatsu, F., Weizhen, J., Kidoya, H., Ishihara, K., Hayashizaki, Y., Nishida, K., Yoder, M.C., Takakura, N., 2018. CD157 marks tissue-resident endothelial stem cells with homeostatic and regenerative properties. Cell stem cell 22 (3), 384–397. https://doi.org/10.1016/j.stem.2018.01.010 e386.

Weinreb, C., Rodriguez-Fraticelli, A., Camargo, F.D., Klein, A.M., 2020. Lineage tracing on transcriptional landscapes links state to fate during differentiation. Science 367 (6479). https://doi.org/10.1126/science.aaw3381.

Werle, S.B., Chagastelles, P., Pranke, P., Casagrande, L., 2019. Hypoxia upregulates the expression of the pluripotency markers in the stem cells from human deciduous teeth. Clin. Oral Invest. 23 (1), 199–207. https://doi.org/10.1007/s00784-018-2427-9.

Willenbring, H., Bailey, A.S., Foster, M., Akkari, Y., Dorrell, C., Olson, S., Finegold, M., Fleming, W.H., Grompe, M., 2004. Myelomonocytic cells are sufficient for therapeutic cell fusion in liver. Nat. Med. 10 (7), 744–748. https://doi.org/10.1038/nm1062.

Wolock, S.L., Krishnan, I., Tenen, D.E., Matkins, V., Camacho, V., Patel, S., Agarwal, P., Bhatia, R., Tenen, D.G., Klein, A.M., Welner, R.S., 2019. Mapping distinct bone marrow niche populations and their differentiation paths. Cell Rep. 28 (2), 302–311. https://doi.org/10.1016/j.celrep.2019.06.031 e305.

Wurmser, A.E., Nakashima, K., Summers, R.G., Toni, N., D'Amour, K.A., Lie, D.C., Gage, F.H., 2004. Cell fusion-independent differentiation of neural stem cells to the endothelial lineage. Nature 430 (6997), 350–356. https://doi.org/10.1038/nature02604.

Xu, X., Li, W.E., Huang, G.Y., Meyer, R., Chen, T., Luo, Y., Thomas, M.P., Radice, G.L., Lo, C.W., 2001. Modulation of mouse neural crest cell motility by N-cadherin and connexin 43 gap junctions. J. Cell Biol. 154 (1), 217–230. https://doi.org/10.1083/jcb.200105047.

Xu, J., Liu, X., Jiang, Y., Chu, L., Hao, H., Liua, Z., Verfaillie, C., Zweier, J., Gupta, K., Liu, Z., 2008. MAPK/ERK signalling mediates VEGF-induced bone marrow stem cell differentiation into endothelial cell. J. Cell Mol. Med. 12 (6A), 2395–2406. https://doi.org/10.1111/j.1582-4934.2008.00266.x.

Yamada, Y., Wakao, S., Kushida, Y., Minatoguchi, S., Mikami, A., Higashi, K., Baba, S., Shigemoto, T., Kuroda, Y., Kanamori, H., Amin, M., Kawasaki, M., Nishigaki, K., Taoka, M., Isobe, T., Muramatsu, C., Dezawa, M., Minatoguchi, S., 2018. S1P-S1PR2 Axis mediates homing of Muse cells into damaged heart for long-lasting tissue repair and functional recovery after acute myocardial infarction. Circ. Res. 122 (8), 1069–1083. https://doi.org/10.1161/CIRCRESAHA.117.311648.

Yan, J., Bao, H., Fan, Y.J., Jiang, Z.L., Qi, Y.X., Han, Y., 2020. Platelet-derived microvesicles promote endothelial progenitor cell proliferation in intimal injury by delivering TGF-beta1. FEBS J. https://doi.org/10.1111/febs.15293.

Yang, M., Arai, A., Udagawa, N., Hiraga, T., Lijuan, Z., Ito, S., Komori, T., Moriishi, T., Matsuo, K., Shimoda, K., Zahalka, A.H., Kobayashi, Y., Takahashi, N., Mizoguchi, T., 2017. Osteogenic factor Runx2 marks a subset of leptin receptor-positive cells that sit atop the bone marrow stromal cell hierarchy. Sci. Rep. 7 (1), 4928. https://doi.org/10.1038/s41598-017-05401-1.

Yoon, C.H., Hur, J., Park, K.W., Kim, J.H., Lee, C.S., Oh, I.Y., Kim, T.Y., Cho, H.J., Kang, H.J., Chae, I.H., Yang, H.K., Oh, B.H., Park, Y.B., Kim, H.S., 2005. Synergistic

neovascularization by mixed transplantation of early endothelial progenitor cells and late outgrowth endothelial cells: the role of angiogenic cytokines and matrix metalloproteinases. Circulation 112 (11), 1618–1627. https://doi.org/10.1161/CIRCULATIONAHA.104.503433.

Yu, Q.C., Song, W., Wang, D., Zeng, Y.A., 2016. Identification of blood vascular endothelial stem cells by the expression of protein C receptor. Cell Res. 26 (10), 1079–1098. https://doi.org/10.1038/cr.2016.85.

Yvernogeau, L., Gautier, R., Petit, L., Khoury, H., Relaix, F., Ribes, V., Sang, H., Charbord, P., Souyri, M., Robin, C., Jaffredo, T., 2019. In vivo generation of haematopoietic stem/progenitor cells from bone marrow-derived haemogenic endothelium. Nat. Cell Biol. 21 (11), 1334–1345. https://doi.org/10.1038/s41556-019-0410-6.

Zengin, E., Chalajour, F., Gehling, U.M., Ito, W.D., Treede, H., Lauke, H., Weil, J., Reichenspurner, H., Kilic, N., Ergun, S., 2006. Vascular wall resident progenitor cells: a source for postnatal vasculogenesis. Development 133 (8), 1543–1551. https://doi.org/10.1242/dev.02315.

Zhang, W., Zhou, X., Yao, Q., Liu, Y., Zhang, H., Dong, Z., 2017. HIF-1-mediated production of exosomes during hypoxia is protective in renal tubular cells. Am. J. Physiol. Ren. Physiol. 313 (4), F906–F913. https://doi.org/10.1152/ajprenal.00178.2017.

Zhang, J., Cui, Y., Li, X., Xiao, Y., Liu, L., Jia, F., He, J., Xie, X., Parthasarathy, S., Hao, H., Fang, N., 2020. 5F peptide promotes endothelial differentiation of bone marrow stem cells through activation of ERK1/2 signaling. Eur. J. Pharmacol. 876, 173051 https://doi.org/10.1016/j.ejphar.2020.173051.

Zhao, M., Tao, F., Venkatraman, A., Li, Z., Smith, S.E., Unruh, J., Chen, S., Ward, C., Qian, P., Perry, J.M., Marshall, H., Wang, J., He, X.C., Li, L., 2019. N-Cadherin-Expressing bone and marrow stromal progenitor cells maintain reserve hematopoietic stem cells. Cell Rep. 26 (3), 652–669. https://doi.org/10.1016/j.celrep.2018.12.093 e656.

Zhou, F., Li, X., Wang, W., Zhu, P., Zhou, J., He, W., Ding, M., Xiong, F., Zheng, X., Li, Z., Ni, Y., Mu, X., Wen, L., Cheng, T., Lan, Y., Yuan, W., Tang, F., Liu, B., 2016a. Tracing haematopoietic stem cell formation at single-cell resolution. Nature 533 (7604), 487–492. https://doi.org/10.1038/nature17997.

Zhou, J., Ghoroghi, S., Benito-Martin, A., Wu, H., Unachukwu, U.J., Einbond, L.S., Guariglia, S., Peinado, H., Redenti, S., 2016b. Characterization of induced pluripotent stem cell microvesicle genesis, morphology and pluripotent content. Sci. Rep. 6, 19743. https://doi.org/10.1038/srep19743.

Zou, T., Dissanayaka, W.L., Jiang, S., Wang, S., Heng, B.C., Huang, X., Zhang, C., 2017. Semaphorin 4D enhances angiogenic potential and suppresses osteo-/odontogenic differentiation of human dental pulp stem cells. J. Endod. 43 (2), 297–305. https://doi.org/10.1016/j.joen.2016.10.019.

Zou, T., Jiang, S., Dissanayaka, W.L., Heng, B.C., Xu, J., Gong, T., Huang, X., Zhang, C., 2019. Sema4D/PlexinB1 promotes endothelial differentiation of dental pulp stem cells via activation of AKT and ERK1/2 signaling. J. Cell. Biochem. 120 (8), 13614–13624. https://doi.org/10.1002/jcb.28635.

Zovein, A.C., Hofmann, J.J., Lynch, M., French, W.J., Turlo, K.A., Yang, Y., Becker, M.S., Zanetta, L., Dejana, E., Gasson, J.C., Tallquist, M.D., Iruela-Arispe, M.L., 2008. Fate tracing reveals the endothelial origin of hematopoietic stem cells. Cell stem cell 3 (6), 625–636. https://doi.org/10.1016/j.stem.2008.09.018.

Glossary

BM: bone marrow
BMEC: bone marrow endothelial cell
CAR: Cxcl12-abundant reticular
CD: cluster of differentiation
CECs: circulating endothelial or endothelial progenitor cells
CFU-Fs: colony forming units-fibroblasts
Cre: CRE (causes recombination) recombinase
CreER/CreERT2/CreERTM: CRE recombinase fused to a mutant form of the human estrogen receptor ligand binding domain
Cxcl12/SDF1: chemokine (C-X-C motif) ligand 12/stromal cell-derived factor 1
E: embryonic day
E-SP: endothelial side population
EC: endothelial cell
ECFCs: endothelial colony forming cells
EGF: epidermal growth factor
EGM: endothelial growth medium
EPC: endothelial progenitor cell
FISH: Fluorescence in situ hybridization
GFP: green-fluorescent protein
GLAST: glutamate-aspartate transporter
GLAST$^+$ Wnt1$^+$ BM-SCs: bone marrow GLAST$^+$ Wnt1-traced stromal cells
HLC: hepatocyte-like cell
hMASCs: human multipotent adult stem cells
HSCs: hematopoietic stem cells
LARRY: lineage and RNA recovery
LepR: leptin receptor
MAPCs: multipotent adult progenitor cells
mMASCs: mouse multipotent adult stem cells
MIAMI: marrow-isolated adult multilineage inducible
MSCs: mesenchymal stem/stromal cells
Muse: multilineage differentiating stress enduring cells
NG2: neuron-glial antigen 2/encoded by CSPG4 gene
P: postnatal day
PDGFR: platelet-derived growth factor receptor
R26R: Rosa 26 reporter strain
S1P: sphingosine-1-phosphate
scATAC-seq: single cell assay for transposition of accessible chromatin-sequencing
Sca-1: stem cells antigen-1/Ly6a
SCF: stem cell factor/Kit ligand
scRNA-seq: single cell RNA-sequencing
SMA: smooth muscle-actin
SP: side population
SSEA: stage specific embryonic antigen-1
SVF: stromal vascular fraction
Tom: tdTomato
VSELs: very small embryonic like stem cells
vWF: von Willebrand factor
Wnt1: wingless-type MMTV integration site family, member 1