Stem cell plasticity: time for a reappraisal?

Roberto M. Lemoli
Francesco Bertolini
Ranieri Cancedda
Michele De Luca
Antonio Del Santo
Giuliana Ferrari
Sergio Ferrari
Gianvito Martino
Fulvio Mavilio
Sante Tura

In recent years an increasing number of publications have claimed that adult mammalian stem cells (SC) may be capable of differentiating across tissue lineage boundaries and that this plasticity may represent a novel therapeutic strategy for tissue regeneration. However, after a first phase of excitement, the issue of somatic SC plasticity remains controversial and the therapeutic perspectives are still elusive. In this review, we examine the general mechanisms which govern the function of SC, the identification and functional characterization of adult SC of different tissues and their putative capacity to transdifferentiate into mature cells of different origin. The potential clinical applications of adult SC for regenerative medicine are also discussed in each chapter. The method employed for preparing this review was the informal consensus development. Members of the Working Group on SC met four times and discussed the single points, previously assigned by the Chairman (S.T.), in order to achieve an agreement on different opinions and approve the final manuscript. All the authors of the present review have been working in the field of SC and have contributed original papers to peer-reviewed journals. In addition to the authors' own work, the present review examines articles published in journals covered by the Science Citation Index and Medline.

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From the Istituto di Ematologia e Oncologia Medica "L. e A. Seràgnoli", Università di Bologna, Bologna, Italia (RL, ST); Divisione di Ematologia, Istituto Europeo di Oncologia, Milano (FB); Laboratorio di Medicina Rigenerativa, Istituto Nazionale per la Ricerca sul Cancro, Genova (RC); Centro per la Ricerca sulle Cellule Staminali Epiteliali, Fondazione Banca del Veneto, Venezia (MDL); Dompè-Biotec, Milano (ADS); Istituto HSR Telethon per la Terapia Genica, Istituto Scientifico H. San Raffaele, Milano (GF); Dipartimento di Scienze Biomediche, Università di Modena-Reggio Emilia, Modena (SF, FM); Unità di Neuroimmunologia, DIBIT, Istituto Scientifico San Raffaele, Milano, Italia (GM).

Correspondence:
Roberto M. Lemoli, MD, Istituto di Ematologia e Oncologia Medica "L. e A. Seràgnoli", via Massarenti 9, 40100 Bologna, Italy.
E-mail: rmlemoli@med.unibo.it

In classical developmental biology, pluripotency and plasticity are considered properties of early embryonic stem cells (ES), while adult stem cells (SC) are traditionally thought to be restricted in their differentiation potential to the progeny of the tissue in which they reside. In higher vertebrates, most adult tissues and organs contain SC capable of self-renewal, proliferation, and differentiation into mature, functional progeny. These SC are more abundant in tissues with a high renewal rate, such as blood, epithelia, or the vasculature, and less abundant in tissues or organs with little renewal capacity, such as myocardial muscle or the central nervous system (CNS). In the last few years, a number of reports from many different groups have claimed a remarkable plasticity in the differentiation potential of SC derived from adult tissues such as the bone marrow (BM), the skeletal musculature, or the CNS. In all cases, differentiation of a SC into a non-canonical progeny (transdifferentiation), e.g., muscle or liver from BM SC, was a rare phenomenon, almost invariably associated with severe damage in the target tissue[1,2] and often with a specific selective pressure for the transdifferentiated progeny.[3] For instance, one type of SC – the neurosphere-derived neural SC - has been reported to turn into a SC with a completely different genetic program – the hematopoietic stem cell (HSC) – under the strong selective pressure of lethal myeloablation.[4] However, some of these reports have not been confirmed in subsequent investigations.[5-7] As an example, the muscle-derived SC reported to give rise to HSC upon transplantation[8] were subsequently shown to be hematopoietic in origin.[9,10] In other cases, cell fusion rather than transdifferentiation was demonstrated to be the main mechanism of the observed plasticity of adult SC.[11-13] Neverthless, the potential plastic properties of adult SC have an obvious relevance for regenerative medicine. The possibility of using SC from easily accessible sources to repair irreversibly damaged tissues, such as infarcted myocardium or cirrhotic liver, or tissues severely damaged by a genetic diseases, such as muscular dystrophy, would have a dramatic therapeutic impact on otherwise untreatable conditions.

Definitions and general concepts about SC

SC are defined as cells with the unique capacity to renew themselves and to give rise to specialized cell types.[14] Therefore, SC have the ability to self-replicate for indefinite periods, perhaps throughout the entire life of the organism. At variance with the large majority of cells of the body that are committed to a specific function, SC are uncommitted and remain as such, until they receive a signal to generate specialized cells. This class of SC is called *pluripotent*, since they have the potential to develop into almost all of the more than 200 different known cell types. SC with this unique property come from embryos and fetal tissue. In 1998, for the first time, investigators isolated this class of pluripotent SC from early human embryos and grew them in culture.[15] Since then, a large body of evidence indicates their pluripotent capacity and their potential to generate replacement cells for a broad array of tissues and organs.

Adult SC are undifferentiated cells that are found in differentiated adult tissues. During the past decade, adult SC have been found in tissues that were not previously thought to harbor them, such as the CNS. More recently, it has been reported that adult SC from one tissue appear to be capable of developing into cell types that are characteristic of other tissues. Thus, the new concept of adult SC developmental plasticity has emerged.[16]

By definition, the only type of *totipotent* SC is the fertilized egg, because it can give rise to all the cells and tissues of the developing embryo. The fertilized egg divides and differentiates until the development of a mature organism. A single pluripotent SC has the ability to give rise to cells originating from all the three germ layers: mesoderm, endoderm, and ectoderm. The only known sources of human pluripotent SC are those isolated and cultured from early human embryos, ES cells, and those isolated from the primordial germ cells of the gonadal ridge of 5- to 10-week fetuses or embryonic germ cells (EG cells). It has recently been described that a subset of adult mesenchymal SC (MSC) are also pluripotent.[17] *Multipotent* SC, such as HSC, can give rise to all blood cells, i.e. cells of different lineages. *Unipotent* SC indicate a cell population, usually present in adult tissues, capable of differentiating along only one lineage.

ES cells

ES cells derive from the inner cell mass of the blastocyst-stage of an embryo, prior to implantation in the uterine wall. ES cells can self-replicate, and give rise to cells derived from all three germ layers. These cells proliferate extensively in the embryo, are capable of differentiating into all adult tissues, and can be isolated and grown *ex vivo*, where they continue to replicate and show the potential to differentiate.[15] Much of the knowledge about ES cells has emerged from applied reproductive biology, i.e., *in vitro* fertilization technologies, and basic research on mouse embryology. ES cell lines can actually be established from virtually all mammals. In humans, blastocysts for the establishment of renewable human ES cell lines may actually be obtained from either supernumerary embryos or from embryos specifically produced for research purposes (*for therapeutic cloning see ref. 18)*. ES cells can be propagated – under certain *in vitro* conditions – almost indefinitely, while maintaining a normal karyotype and totipotency, as has recently been shown by culturing ES cell lines in the presence of leukemia inhibitory factor (LIF). Cultures of human pluripotent SC have active telomerase, indicating that they have the ability to replicate for many generations.

Current challenges for the therapeutic use of ES cells are to direct their differentiation into specialized cell populations, and also to devise strategies to control their development or proliferation once infused *in vivo*. Although a detailed discussion of the properties of ES cells is not the aim of this review, the main characteristics defining ES cells are listed in Table 1.

Adult SC

Adult SC (Table 2) have long-term self-renewal capacity and can give rise to mature cell types with specialized functions. Typically, SC generate intermediate cell types (progenitors and more differentiated precursors) before they achieve their fully differentiated state. Progenitors and precursor cells are usually regarded as *committed* to differentiate along a specific cellular pathway. The primary function of SC is to maintain homeostasis, and, with limitations, to replace cells dying because of injury or disease. Adult SC behave very differently depending on their local environment and tissue origin. HSC are located in the BM where they differentiate into mature blood cells. Only 1 in 10,000 to 15,000 BM progenitors is a HSC.[14] Conversely, epithelial SC in the small intestine reside at the bases of crypts, deep invaginations between the mature, differentiated epithelial cells that line the lumen of the intestine. These epithelial crypt cells divide fairly often, but remain part of the stationary group of cells they generate.[19]

In order to be classified as an adult SC, the cell should be capable of self-renewal for the lifetime of the organism. This criterion, although fundamental to the nature of a SC, is difficult to prove *in vivo*. It is nearly impossible, in an organism as complex as a human, to design an experiment that will allow the tracking of candidate SC over the entire lifetime. In practice, a candidate SC must repopulate the tissue upon transplantation. An adult SC should also be able to give rise to fully differentiated cells that have

Table 1. Properties of ES cells.

Tissue origin	Derived from the inner cell mass/epiblast of the blastocyst.
Long-term self-renewal	Capable of undergoing an unlimited number of symmetrical divisions without differentiating.
Karyotype	Exhibit and maintain a stable, full (diploid), normal complement of chromosomes.
Pluripotentiality	Pluripotent ES cells can give rise to differentiated cell types that are derived from all three primary germ layers of the embryo (endoderm, mesoderm, and ectoderm). Capable of integrating into all fetal tissues during development. Mouse ES cells maintained in culture for long periods can still generate any tissue when they are reintroduced into an embryo to generate a chimeric animal. Capable of colonizing the germ line and giving rise to egg or sperm cells.
Clonogenicity	A single ES cell can give rise to a colony of genetically identical cells, or clones, which have the same properties as the original cell. ES cells express the transcription factor Oct-4, which then activates or inhibits a host of target genes and maintains ES cells in a proliferative, non-differentiating state.
Cell Fate	Can be induced to continue proliferation or to differentiate.
Cell Cycle	Lack the G1 checkpoint in the cell cycle. ES cells spend most of their time in the S phase of the cell cycle, during which they synthesize DNA. Unlike differentiated somatic cells, ES cells do not require any external stimulus to initiate DNA replication.

Table 2. Properties of Adult SC.

Tissue origin	Present in many tissues.
Long-term self-renewal	Capable of maintaining homeostasis of the SC compartment for the entire lifetime of the organism.
Karyotype	Exhibit and maintain a stable, full (diploid), normal complement of chromosomes.
Potentiality	The large majority of adult stem cells are not pluripotent, like ES, since they have a limited differentiation capacity. They can be multi-potent, such as hematopoietic SC or unipotent such as skin SC. Experimental evidence suggests that the only exception are MAPc since these can give rise to differentiated cells of all the three types of primary germ layers of the embryo (endoderm, mesoderm, and ectoderm).
Clonogenicity	A single adult SC, *in vitro*, can only give rise to a colony of differentiated cells lacking the properties of the original cell The molecular mechanisms that maintain adult SC cells in a proliferative, non-differentiating state are almost completely unknown.
Cell Fate	Can be induced to differentiate.
Cell Cycle	The large majority of adult SC are in a quiescent state. Adult SC require an external stimulus from the microenvironment to enter the cycle and initiate DNA replication (stem cell niche).
Plasticity	Adult SC may have the ability to generate specialized cells of other tissues. The mechanism is still debated (cell fusion ? transdifferentiation ?)

Table 3. Adult SC types and their developmental plasticity.

Tissue of origin	*Cell type*	*Species*	*Tissue damage*	*Tissue formed In vitro*	*In vivo*
Muscle	Satellite cells	–	–	–	–
Skin	Epithelial SC	–	–	–	–
CNS	Neural SC	Mice	TBI	–	BM, Muscle
Liver	Hepatocytes, oval cells°	Rat	STZ*	Pancreas	Pancreas
Kidney	Renal SC	–	–	–	–
	MSC	Human	–	BM, Liver	–
Pancreas	Pancreatic SC	Rat	–	Liver	–
Heart	Cardiac SC	Mice	TBI	–	Endothelium

*Example of developmental plasticity of SC types discussed in this review. The frequency of transdifferentiation to tissues different from that of origin, in vivo is either minimal or not present. Endothelial SC are included in Table 4 within BM-derived SC. For appropriate references see the text; *STZ: streptozotocin; °Only oval cells showed the capacity to generate pancreatic endocrine hormone-producing cells, in vitro and in vivo.*

mature phenotypes, are fully integrated into the tissue, and are capable of specialized functions that are appropriate for the tissue.

Adult SC have been identified in many animal models and human tissues. The list of adult tissues reported to contain SC is growing and includes BM, peripheral blood, brain, spinal cord, dental pulp, blood vessels, skeletal muscle, epithelia of the skin and digestive system, cornea, retina, liver, pancreas, heart, and the CNS (Table 3 and 4).

The most abundant information about adult human SC comes from studies on the BM and blood. There are two major types of SC found in the BM (Table 4): HSC which generate blood cells, and MSC that support hematopoiesis and can differentiate into multiple tissues of the three germinal layers (*see below*).

Adult SC regulation

During the process of differentiation, the proliferative potential of adult SC is progressively lost while specific differentiation features are acquired. According to this hierarchical model, the most primitive hematopoietic SC are quiescent, residing in the G_0 phase of the cell cycle, and are protected from depletion and exhaustion. Quiescent SC enter the cell cycle either randomly or under the influence of micro-environmental stimuli,[20] such as growth factors or cytokines mainly produced by accessory or differentiated cells of the tissue.

During their transit through the cell cycle, SC exert their function. These observations might be explained by alterations in chromatin structure during cell cycle progression that could have marked effects on gene

Table 4. BM-derived SC types and their developmental plasticity.

	Cell type	Species	Tissue damage	Tissue formed		Frequency (%)
				In vitro	*In vivo*	
MAPc		Mice, Rat, Human	None or TBI	Multiple tissues of the 3 germinal layers	Multiple tissues of the 3 germinal layers	0-12.5
MSC*		Mice, Rat, Human	None or TBI	Chondroblasts Skeletal muscle Osteoblasts Candroblasts Adipocytes Neural Cells	Chondroblasts Skeletal muscle Osteoblasts Candioblasts Adipocytes Neural Cells	
	Unfractionated	Mice	TBI		Pancreas	1.7-3
	Unfractionated	Rat	CCl_4		Liver	0.14
	Unfractionated	Human (BM,PB)	TBI		Liver	
					Skin	5-7
					Intestinal	2-6
					Epithelium	4-6
HSC	Unfractionated°	Human, Mice	TBI		CNS	
	Unfractionated (Purified)	Mice	TBI ± genetic deficiency (mdx mice)		Skeletal muscle	From rare to <10
	Unfractionated°	Mice, Dog	Ischemia ± TBI		Cardiac muscle ± vasculature	2-25
	Unfractionated	Mice, Human	TBI		Liver	2.2
	c-Kit	Mice	STZ		Pancreas	14
	Sca +	Mice	Ischemia		Kidney	2-21
	KTSL°	Mice	TBI + FAH		Liver	rare
	CD34 +	Human ?Mice	TBI ± CCl_4		Liver	–
	CD34 +	Mice	Ischemia		Vasculature	20-25
	Homed SC	Mice	TBI		Multiple epithelial tissues	0.19-20
	Monocyte precursor°	Mice	TBI		Skeletal muscle	From rare to < 10
	SP°	Mice, Human	TBI ± Ischemia		Liver, cardiac, muscle, endothelial cells	0.02-4
	SP°	Mice	TBI ± genetic deficiency (mdx mice)		Skeletal muscle	1-10
	c-kit+ Lin -°	Mice	Ischemia		Cardiac muscle and vasculature	54

*TBI: Total-body irradiation; KTSL, c-Kit$^+$,Thy-1^{Low}, sca-1+ and lineage markers -; STZ, streptozotocin; "HOMED HSC", single HSC that homed to the BM after transplantation and not divided progenitor cells; MSC: mesenchymal SC; SP: side population; FAH: fumarylacetoacetate; MAPc: multipotent adult progenitor cells. *MSC with capacity to differentiate to tissues different from that of origin were also found in the kidney, adipose tissue, dental pulp, and CNS. °Cell fusion mechanism was demonstrated. For appropriate references and details see the text.*

expression and therefore on cell fate. A decreased concentration of growth factor when stem or progenitor cells are actively proliferating can lead to a cell cycle arrest in G_1 and to the possibility that these arrested cells activate different genetic programs such as differentiation, apoptosis or reverse cell cycle progression toward G_0. Many data exist to support such a hierarchical model: (i) marrow cells have been separated based on their repopulating potential and progenitors have been characterized as exclusively committed to the production of restricted progeny; (ii) expansion of different progenitor types in cytokine-stimulated *in vitro* culture with loss of long-term engraftment capacity demonstrates the existence of a progenitor hierarchy, at least of the more differentiated progenitor cells.

However, this hierarchical model has been challenged.[21] In fact, rather than hierarchical transition from SC to progenitor cells, it appears that a fluctuating continuum exists, in which the phenotype of SC shifts from one state to another and back depending on the cells' kinetic state. These phenotypic shifts are based on chromatin remodeling associated with cell cycle transit and particularly with the G_1 phase of the cycle, which reversibly alters the surface phenotype of SC determining their response to environmental stimuli. According to this model of competence and progression, studied in fibroblasts, quiescent SC may proliferate and maintain their renewal capacity and then return to G_0 due to a drastic drop in growth factor concentration.[22] This mechanism can explain the maintenance of the SC pool with kinetically quiescent cells. On the other hand, it is possible that cell cycle transit, particularly in the G_1 phase, in which gene transcription is much more efficient, is associated with a con-

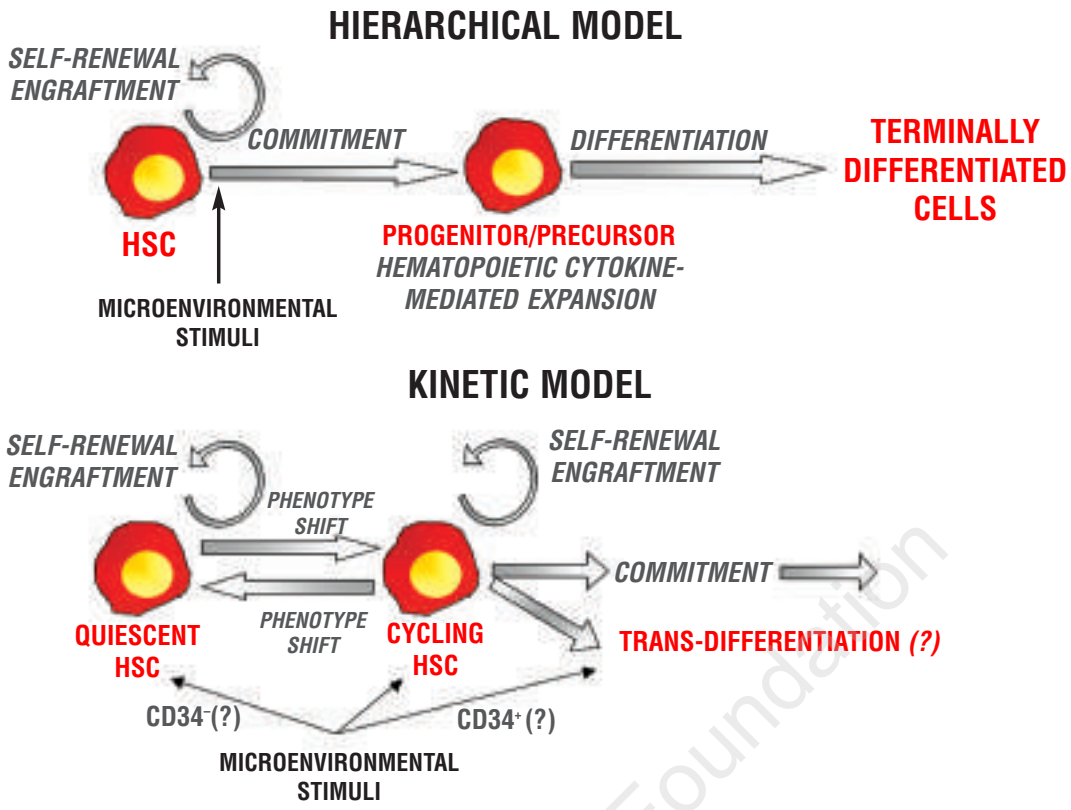

Figure 1. Hierarchical and kinetic models: transdifferentiation of marrow SC to non-hematopoietic cells in different tissues.

tinuously changing SC phenotype depending on different genetic programs activated by microenvironmental stimuli that differ in the different areas of the tissue and therefore lead to SC commitment, differentiation, apoptosis or to developmental plasticity. In fact, one can speculate that such flexibility might also explain the recently described transdifferentiation of marrow SC to non-hematopoietic cells in different tissues (Figure 1). In conclusion, according to this model, the kinetic status of SC is very important since only proliferating SC can move backward to the G_0 phase of the cell cycle, undergo differentiation, apoptosis or transdifferentiation.

Identification of SC in adult tissues and models of differentiation across lineage boundaries (developmental plasticity)

Pancreatic SC and models of transdifferentiation into pancreatic tissue

Several experimental models have demonstrated that both the endocrine[23,24] and the non-endocrine[25,26] compartments of the pancreas contain multipotent progenitors capable of differentiating into pancreatic islet-like structures. Moreover, recent data demonstrate that adult pancreatic insulin-producing cells are capable of replicating themselves.[27] However, the presence of additional sources of pancreatic precursor cells has recently been reported. Murine and human pluripotent ES cells have been shown to differentiate into insulin-producing cells with the ability to cure diabetic mice.[28] Furthermore, adult hepatic oval SC have demonstrated the capacity to generate functional endocrine pancreas under culture conditions similar to those used to differentiate pancreatic SC into insulin-secreting, cells.[29] On the other hand, pancreatic SC possess the ability to differentiate into liver cells.[30] These findings, coupled with the notion that both the liver and the ventral pancreas seem to develop from the same cell population within the embryonic endoderm, strongly suggest that liver and pancreas share common SC whose differentiation is determined by their location, by local growth factors, glucorticoids or by the induction of the transcription factor C/EBPβ, which is the the key factor dictating hepatic cell differentiation from terminally differentiated pancreatic elements.[30] Recently, two different groups have reported that BM-derived SC can differentiate into pancreatic islet cells.[31,32] In the first study,[31] murine male green fluorescent protein positive (GFP^+) unselected BM cells were transplanted into irradiated female recipients. After 4 to 6 weeks from transplant, GFP^+, Y-chromosome$^+$ and insulin$^+$ cells were detected

in the islets. Molecular analysis showed the expression of pancreas islet markers such as insulin I, II, GLUT-2, IPF-I, HNF1a, HNF-1b and PAX6 in 1.7-3% of analyzed cells. Functionally, the BM-derived pancreatic cells showed a physiological behavior typical of β cells by secreting insulin upon stimulation with glucose or the glucagon-like peptide exendin. In the same paper, the authors ruled out that cell fusion between BM and pancreatic cells had occurred. To test this issue, female *stop-lox GFP* mice, in which GFP is expressed after recombination by cre recombinase, were transplanted with male BM cells expressing cre recombinase. The fact that Y^+, GFP-negative pancreatic cells were found suggests that no fusion had occurred.[31,32] Hess *et al*.[32] transplanted c-kit^+ BM cells into recipient mice with streptozocin-induced pancreatic damage. Using this model of chemical injury, the authors demonstrated the capacity of partially purified BM SC to differentiate, at low frequency, into fully functional ductal and islet structures. These studies underline the concept that different methods should be combined to obtain reliable analysis of insulin-expression by pancreatic cells. A recent report[33] demonstrates that ES cells can be positively stained with antibodies to insulin taken up from the culture medium.

Kidney SC and evidence that BM SC contribute to repair injured renal tissue

Epithelial cells within the renal tubules are believed to represent the kidney SC population as they dedifferentiate to a more mesenchymal phenotype and then proliferate in response to acute tubular necrosis to repair the injured tissue.[34] Kidney progenitor cells, capable of undergoing complete nephrogenesis and potentially suitable for transplantation, can also be isolated from human embryos of 7 to 8 weeks.[35] Recent data indicate that human BM cells might contribute to the generation of both epithelial[36,37] and non-epithelial[38-40] cell types in the kidney. The presence of Y-$chromosome^+$ epithelial renal cells in males who had received a kidney transplant from a female donor was shown, thus suggesting an alternative source of kidney SC. Kale *et al*. have used a model of ischemic renal damage to demonstrate the ability of murine BM-derived SC (Lin-Sca^+) to mobilize in response to ischemic injury and to differentiate into renal tubular cells within 48 hours in order to contribute to tissue repair.[41] After surgical clamping of the renal artery followed by reperfusion, up to 20.9% of outer medulla renal tubules contained BM-derived cells which contributed to the significant reduction of blood urea nitrogen as compared to that in mice which did not receive BM cells. Interestingly, Lin^+ cells (i.e. cells already committed to the lympho-hemopoietic lineage) did not show differentiation into epithelial cells.

BM to liver

In contrast to other regenerating tissues, liver regeneration after tissue damage is not dependent on only one class of progenitor/stem cells.[42] At least two resident cell populations have been shown to act as putative SC. Whereas moderate cell loss is restored by mature hepatocyte self-renewal, more severe liver injury induces the activation of a *facultative* SC compartment located within the smallest branches of the intrahepatic biliary tree, the so-called *oval cells* which give origin to hepatocytes and the bile duct epithelium. Of note, liver oval SC express the antigens CD34 and c-Kit, which are strongly associated with the hematopoietic stem/progenitor cell phenotype.[43] At the time of writing, a few patients have been succesfully transplanted with allogeneic hepatocytes to correct inherited liver-based metabolic disorders.[44,45] These preliminary results suggest that transplantation of less than 5% of total hepatic mass induces at least a partial correction of the metabolic abnormalities.

Recently, a third source of liver-repopulating cells has proven to be the BM. Petersen *et al*.[46] transplanted syngeneic male BM cells into lethally irradiated female recipient rats that had been treated with 2-acetylaminofluorene, to inhibit hepatocyte proliferation, and then received hepatic injury by carbon tetrachloride (CCl_4) to induce oval cell activation. Donor-derived, Y-$chromosome^+$ oval cells and mature hepatocytes were found 9 and 13 days after liver damage, respectively. Additional evidence of a BM-derived hepatic SC came from Theise *et al*.[47] who also used a gender mismatch BM transplantation strategy to track donor-derived SC in the liver of lethally irradiated mice. This study provided at least two important findings: (i) BM may contribute to normal renewal of liver tissue in the absence of obvious damage; (ii) regeneration of hepatic tissue could be accomplished by HSC since 200 $CD34^+Lin^-$ cells produced the same degree of engraftment (1-2% at 6 months) as did 20,000 unseparated BM cells. Two further studies[48,49] demonstrated that, also in humans, hepatocytes can derive from BM. Both groups examined the livers of female patients who had received a BM transplant from a male donor and female livers transplanted into male recipients which had to be removed for recurrent disease. Using *in situ* hybridization for Y-chromosome analysis, 4 to 43% hepatocytes and 4 to 38% cholangiocytes were found to be of extrahepatic origin.[49] Taken together, these studies provided the *proof of principle* that BM contributes to liver regeneration. Further investigations were then addressed to important issues such as the functional capacity of marrow-derived hepatic cells, the BM cell type involved in liver tissue differentiation, the molecular mechanisms regulating the recruitment of marrow cells into liver and finally some studies challenged the concept of SC plasticity itself.

Lagasse *et al*.,[3] used BM transplantation in female recipients deficient in the enzyme fumarylacetoacetate hydrolase (FAH-/-), an animal model of fatal hereditary tyrosinemia type I. FAH-deficient mice die after birth unless rescued with NTBC, a compound that prevents the accumulation of toxic metabolites in the tyrosine catabolic pathway. Liver function was restored by transplantation of 10^6 unfractionated wild-type ($FAH^{+/+}$) BM cells or by 50 highly purified hematopoietic c-$Kit^+Thy^{+-}Lin^-Sca\text{-}1^+$ SC. Interestingly, only HSC, and not committed precursors, showed the capacity to differentiate into hepatocytes and to rescue an otherwise lethal metabolic disease. More recently, Wang *et al*.[50] transplanted purified human hematopoietic $CD34^+$ or $CD34^+CD38^-CD7^-$ SC into non-obese diabetic, severe combined immunodeficiency (NOD/SCID) and NOD/SCID $\beta_2$microglobulin (β_2M)-null mice. One month after the transplant, CCl_4 was given to mice to induce severe liver damage and hepatocyte proliferation. Four weeks later, the livers were harvested and the expression of mRNA for human albumin and CK19, a marker of bile duct cells, was found. Human albumin was also found in the serum of mice and the results were improved by the administration of hepatocyte growth factor (HGF).[50] Taken together, these studies provide strong evidence that both murine and human HSC can generate fully functional hepatic tissue. Furthermore, it was demonstrated that even a single $CD34^+Lin^-Sca^+$ cell can give rise (with variable efficiency) to epithelial cells in different organs of recipient animals, including <1% of biliary cells.[1] A non-hematopoietic population of mesenchymal multipotent adult progenitor cells (MAPc), which can differentiate into multiple lineages, has been isolated from the BM.[17] Murine and human MAPc can differentiate into functional hepatic tissue *in vitro* producing albumin and urea and capable of storing glycogen.[51] Thus, different cellular types from the BM or the peripheral blood[52] of hematopoietic[1,3,46,53,54] and non-hematopoietic origin[17,51] share the capacity to generate hepatocytes. This finding supports the concept that *plasticity* may not be restricted to a unique SC population but, rather, may be a general property of marrow cells that redirect their transcriptional program under appropriate stimuli. In this view, the so-called BM *side population* (SP), which is characterized by its capacity to efflux the Hoechst 33342 dye, is capable of engrafting into the damaged liver and to contributing to liver repair.[55] Interestingly, SP $CD34^{low/negative}$ marrow SC share many functional properties, including the potential for differentiation into tissues other than the original one, with SP SC found in skeletal muscle and liver.[55]

Very recently, the mechanisms leading to the homing of HSC to the liver have been investigated.[56] The chemokine stromal cell-derived factor-1 (SDF-1), which is a pivotal molecule for SC migration and BM homing, was found to be expressed after irradiation of liver bile duct epithelium of NOD/SCID mice; furthermore, its expression was upregulated by hepatic CCl_4-mediated damage. Human $CD34^+CD38^-$ HSC transplanted into immunocompromised mice migrated to the liver whereas the inhibition of SDF-1 receptor CXCR4 abolished their engraftment. HGF and stem cell factor, which are secreted after liver injury, increased the expression of CXCR4 and SDF-1 thus augmenting the recruitment of marrow cells to the liver.[56] This study underlines the potential of $CD34^+$ cells to migrate in response to stress signals in order to repair non-hematopoietic tissue. However, when the kinetics of liver repopulation after BM transplantation was studied, Wang *et al*.[57] found that the appearance of BM-derived hepatocytes was quite slow and inefficient with the first cells being detectable after 2 months with a frequency of only 1/150,000 which is below any therapeutic threshold. Finally, it should be mentioned that two recent studies[11,12] have challenged the concept of SC transdifferentiation potential by showing that cell fusion is the main mechanism by which HSC acquire the function of mature hepatocytes. Therefore, investigators should test, when possible, whether cell fusion is responsible for the reprogramming of the gene expression pattern and function of adult SC. In this view, Harris *et al*.[58] using the Cre/lox system in a transplant model and Jang YY *et al*.[59] *in vitro* and *in vivo*, have shown that HSC can convert into liver,[58,59] lung, and skin cells[58] without fusion. Thus, it may be possible that cell fusion occurs on the basis of the type of damage. Whereas FAH mice are rescued by a cell fusion mechanism, true differentiation of HSC into liver cells takes place after irradiation.[58]

Muscle SC

Adult skeletal muscle fibers harbor mononucleated quiescent precursor cells called satellite cells. These cells are located along the muscle fibers, between the sarcolemma and the basement membrane, and contribute myonuclei to growing fibers during postnatal growth and to regenerating fibers after tissue injury.[60] Satellite cells are also capable of self-renewal, but their number, which is highest in postnatal muscle, declines with age. During adult life, satellite cells remain quiescent and once they become activated by injury they start to express myogenic regulatory factors, as do muscle precursors during skeletal muscle development. Until recently, the identification and the isolation of satellite cells was quite difficult, since it was based on electron microscopy detection. The discovery of proteins differently expressed by satellite cells and myofibers has contributed to their isolation and functional characterization.[61]

The myogenic potential of adult HSC

The first demonstration of non-canonical differentiation of adult SC came from studies showing that BM-derived cells participate in the repair of damaged muscle upon transplantation into irradiated recipient mice.[62] Subsequent reports have shown that BM-derived cells[63] or SC-containing fractions[64] are recruited to myogenic differentiation and restore dystrophin gene expression in a murine model of dystrophin-deficient (Duchenne's) muscular dystrophy. The origin of the BM-derived myogenic cells and their physiological role, if any, in the homeostasis of muscle tissue, are still uncertain. The existence of a circulating myogenic progenitor cell derived from the BM is not predicted on the basis of classic embryology, which ascribes the origin of all skeletal myogenic cells to the somites. One possibility is that BM-derived myogenic cells originate from multipotent MSC located in the BM stroma. However, recruitment of MSC or MAPc to myogenesis has never been observed *in vivo*. On the other hand, transplantation of purified hematopoietic cell fractions indicate that cells endowed with myogenic potential *in vivo* derive from the hematopoietic rather than from the stromal fraction of the BM.[64-66] However, it remains to be defined whether SC directly or their differentiated progeny are recruited to the muscle and induced to myogenic differentiation, and whether HSC transdifferentiate into a muscle-specific stem/progenitor cell (e.g. a satellite cell), or directly into a terminally differentiated progeny (i.e. the muscle fiber).

Very recent data, based on the use of hybrid LysM-Cre/ ROSA$^{flox/STOP}$ transgenic mice, suggested a role for the myeloid lineage in the generation of newly formed myotubules.[67] However, the rigor of this finding is challenged by the demonstration that the LysM promoter is indeed active in fully pluripotent HSC.[68] A better definition of the cells capable of changing fate in response to these signals, as well as a better knowledge of the signals themselves, is therefore needed in order to understand the blood-to-muscle transition fully. Expansion of transplantable hematopoietic cells and active recruitment to myogenic differentiation are in fact critical factors for a possible use in cell/gene therapy of muscular dystrophy.

SC transplantation for muscular dystrophy: a therapeutic perspective?

Duchenne's muscular dystrophy (DMD) is due to the total or partial deficiency of dystrophin, a component of the multi-protein complex linking the cytoskeleton of the muscle fiber to the extracellular matrix. In the absence of dystrophin, the complex is functionally impaired, and the mechanical stress associated with contraction progressively leads to degeneration of skeletal muscle fibers, muscle wasting, progressive impairment of movements, and eventually paralysis and death. The current therapeutic approaches to DMD involve pharmacological control of inflammation, which achieves beneficial effects but has no impact on the life expectancy of the patients. Gene therapy of DMD is based on the concept of providing a copy of a normal dystrophin cDNA to muscle fibers or satellite cells, *in vivo* or *ex vivo*. The discovery that transplantable, BM-derived cells may give rise to muscle tissue under certain experimental circumstances has obviously raised much hope for a new form of cell-based therapy of DMD.

From the clinical point of view, however, the studies on *mdx* mice have been disappointing. They showed that after BM transplantation less than 0.5% of the muscle fibers of the recipient mice are reconstituted by a donor cell source, with no practical impact on the murine pathology. The implication of these findings is that either the efficiency of the myogenic conversion is too low to be practically useful, or that the *mdx* background does not provide enough differentiation stimuli, or selective advantage, to hematopoietic cells to trigger their expansion and the replacement of the resident muscle cells.

More recently, a possible alternative to BM-derived primary cells has been developed in the murine system. This is represented by blood-vessel associated, multipotent progenitor cells – the so-called mesoangioblasts – which have the capability to differentiate into muscle tissue *in vivo* with a relatively high efficiency. Mesoangioblasts are derived in culture by clonal expansion of embryonic or fetal aorta-derived mononuclear cells, and have the remarkable property of homing and differentiating into muscle fibers when delivered to a muscle through the arterial circulation. Intra-arterial delivery of genetically-modified mesoangioblasts led to repopulation and massive replacement of sarcoglycan-deficient fibers in a murine model of limb-girdle muscular dystrophy.[69] This study indicates that mesoangioblasts that have been expanded *in vitro* from an easily accessible source may be genetically modified and used as a combined gene/cell therapy to treat muscular dystrophy.

The role of endothelial stem/progenitor cells (EPC) in health and disease

Until recently, it was thought that vasculogenesis (EPC-derived generation of new vessels) is restricted to the period of embryonic development, whereas in the adult only angiogenesis (sprouting from mature endothelial cells) contributes to the generation of new blood vessels.[70] During antenatal life, EPC and HSC are thought to derive from a common progenitor, the hemangioblast.[71] In adult life, there is increasing evidence that HSC provide functional hemangioblast activity (*see below*).

EPC may persist in the marrow and circulate in the blood during adult life.[72] In fact, recent preclinical and clinical studies have indicated that EPC can restore tissue vascularization and that the growth of some neoplastic diseases may depend on EPC-induced vasculogenesis rather than on angiogenesis.[73]

The presence of marrow-derived EPC able to participate in vasculogenesis was first demonstrated in 1998 by Shi *et al*.[74] in a canine bone marrow transplantation model: when a Dacron graft was implanted 6-8 months after transplantation, only donor endothelial cells colonized the vascular prosthesis. In a transgenic mouse model expressing a marker gene under the transcriptional regulation of an endothelial-specific promoter (*Flk-1* or *Tie-2*), marrow-derived EPC were found to generate new vessels in the endometrium after ovulation, in implanted colon tumors, in cutaneous wounds and in neovascularization foci at the border of myocardial infarcts generated by coronary ligation.[75] Circulating EPC have been detected in the blood after vascular injuries, vascular trauma induced by burn injury, or in neoplastic patients.[73] In fact, vascular trauma and organ regeneration may result in the release of EPC-mobilizing chemokines and/or VEGF-A. This latter molecule, is currently considered the single most potent growth factor promoting ESC mobilization in a complex mechanism mediated through activation of metalloproteinases (MMP) and adhesion molecules.

EPC can be distingushed from the larger population of circulating mature endothelial cells (CEC) by the expression of CD133 antigen. In addition to the marrow, it seems possible that the parenchyma of the systemic vasculature or certain organs may host embedded EPC. For example, EPC isolated from the skeletal muscle accordingly to the side population phenotype can differentiate into mature endothelial cells.[76]

The role of EPC in revascularization

Retinal and brain revascularization

Diabetes and age-related prematurity and macular degeneration are associated with abnormal compensatory choroidal and retinal neovascularization, which, in turn, can lead to proliferative retinopathy. SC have been shown to contribute to retinal neovessel formation. In murine studies, a single GFP-expressing $Sca1^+c\text{-}Kit^+Lin^-$ cell injected into lethally irradiated mice resulted, after ischemia-induced laser occlusion, in the incorporation of donor-derived GFP^+ endothelial cells into retinal neovessels.[77] In another study, Lin^- progenitors were intravitreally injected into neonatal and adult mice with photocoagulated retinas. Surprisingly, Lin- cells were localized into the astrocytes and formed retinal vessels. This study indicated that astrocytes may promote the differentiation and incorporation of marrow–derived EPC into functional vessels.[78]

Neovascularization near the ischemic boundary zone is frequently observed after a cerebral infarction. Transplantation of male-derived donor marrow cells into female mice, who were later subjected to middle cerebral artery occlusion, has been associated with a predominance of donor-derived cells in the vasculature repair site 3 days after occlusion.[79]

Graft revascularization

Autologous vascular grafts or a decellularized matrix are used to replace altered coronary arteries. Thrombus generation on the matrix-blood contact surface is one of the most frequent complications. EPC can generate non-thrombogenic mature vascular cells to coat decellularized or biodegradable surfaces. After the pioneering canine study by Shi *et al*.,[74] marrow cells have been embedded into synthetic prostheses before grafting into the aortas of dogs, resulting in generation of a non-thrombogenic endothelialized surface. Autologous ovine EPC were also seeded on decellularized vessels and grafted into carotid arteries to generate non-thrombogenic functional vessels that remained patent with no occlusions for 120 days, in contrast to the endothelial-free vascular grafts that formed thrombi.[80] Thus, graft precoating with EPC-derived mature endothelium may facilitate *in vivo* remodeling.

Limb ischemia

Autologous marrow cells injected into ischemic gastrocnemius muscle restored limb function in preclinical studies of peripheral arterial disease and in preliminary clinical observations.[81] Given the use of crude marrow cell suspensions, it remains to be determined whether these results were due, at least in part, to hematopoietic cells. It should be noted, however, that revascularization of ischemic limbs depends on the balanced formation of blood and lymphatic vessels, because lymphatic dysfunction results in edema and non-healing ulcers. Overexpression of VEGF-C has accelerated functional recovery of ischemic limbs by enhancing lymphangiogenesis and angiogenesis and decreasing edema formation.[82]

Myocardial regeneration and revascularization

It has been reported that marrow cells may differentiate into myogenic and vascular cells in a process defined as angiomyogenesis. In murine studies on infarcted animals, mobilized $VEGFR2^+$, $CD117^+$ cells homed to the infarcted tissue and contributed to neoangiogenesis.[83] In other studies, marrow–derived $CD117^+$ or side population cells injected into the infarction site differentiated into myocardial cells and mature endothelium.[84-86] Furthermore, injection of human $CD34^+$ or autologous $CD31^+$ marrow cells into rats increased vessel density during ameroid-induced

blood vessel constriction and ischemia.[87,88] In a very interesting recent study, the injection of marrow cells transduced with the prosurvival gene *Akt1* (encoding the Akt protein) into ischemic rat myocardium inhibited cardiac remodeling by reducing intramyocardial inflammation, collagen deposition and cardiac myocyte hypertrophy, and regenerated most of the lost myocardial volume, and completely normalized systolic and diastolic cardiac function.[89] Taken together, these preclinical studies suggest that direct introduction into a permissive infarcted or ischemic myocardium may allow progenitor cell incorporation, neoangiogenesis and improvement of cardiac function. In pioneering human clinical studies, crude autologous marrow cell suspensions have been delivered by arterial catheters into coronary arteries to repair infarcted and ischemic tissues by transendocardial injections or guided electrochemical mapping.[90-92] In other settings, the clinical efficacy of *ex vivo* expanded peripheral blood mononuclear cells was compared to that of marrow–derived EPC in restoring revascularization after acute myocardial infarction.[93] Interestingly, some investigators have also studied the injection of purified, marrow-derived $CD133^+$ cells into the infarcted or ischemic areas, with or without concomitant coronary artery bypass grafting. Blood flow and ventricular function improvements observed in these patients indicate that such procedures are feasible, safe and, possibly, clinically effective.[94] A first randomized clinical trial has demonstrated that, in patients affected by myocardial infarction, intracoronary-injected autologous marrow cells can improve the ejection fraction evaluated six months after a percutaneous coronary intervention.[95] However, though neoangiogenesis from BM-derived SC seems to be well established, the capacity of HSC to transdifferentiate into cardiomyocytes is still debated.[96,97] In addition, recent experimental and clinical data[98,99] have shown restenosis of coronary arteries following BM SC therapy, thus suggesting that the route of administration of BM cells may be important for cellular therapy.

Two recent reports have proposed the existence of adult heart-derived cardiac progenitor cells. Oh *et al.*[100] identified a murine population of *cardiac stem cell candidates* expressing Sca-1. Initially, these cells expressed neither cardiac structural genes nor Nkx2.5 but they differentiated *in vitro* in response to 5'-azacytidine, in part depending on Bmpr1a, a receptor for bone morphogenetic proteins. Given intravenously after ischemia, these cells engrafted in injured myocardium. By using a Cre/Lox donor/recipient pair, differentiation was shown to occur almost equally, with and without fusion to host cells. Beltrami *et al.*,[101] have reported the existence of Lin^- $c\text{-}kit^+$ cells with the properties of cardiac SC. These cells are self-renewing, clonogenic, and able to generate myocytes, smooth muscle, and endothelial cells. When injected into an ischemic heart, these cells (or their clonal progeny) were able to reconstitute well-differentiated myocardium and new vessels.

Neural SC and their use as a therapeutic tool in neurological disorders

Neural SC (NSC) are considered a heterogeneous population of mitotically-active, self-renewing, multipotent cells of both the developing and the adult nervous system showing complex patterns of gene expression that vary in space and time. Early in the 1960s, neural cells behaving as SC entities were isolated from the embryonic mammalian central as well as peripheral nervous system.[102,103] Since then, SC have been isolated from virtually the entire adult CNS and regions such as the subventricular zone (SVZ) of the lateral ventricles, the dentate gyrus and the cerebral cortex have been shown to contain stem-like cellular elements.[104] During development, the frequency of NSC decreases over time, while the activation of cellular programs of differentiation into lineage (neuronal or glial)-restricted precursors increases.[105] In the adult CNS, stem-like cells – although showing modest proliferation characteristics – are capable of driving neurogenesis in specialized regions of the brain (i.e. the olfactory bulb, the hippocampus, the SVZ, the central canal of the spinal cord), which behave as highly specialized tissue niches. On the other hand, cycling progenitor cells of apparent glial lineage, distributed throughout the whole brain parenchyma, drive adult gliogenesis.

Independently of the accepted terminology – SC, precursors or progenitors – the origin of this discrete population of undifferentiated neural cells is still debated. So far, two principal theories have been postulated. One theory claims that the true NSC of the adult SVZ differentiate from ependymal cells expressing the intermediate filament protein nestin,[106] while the other theory identifies NSC as slowly dividing astrocyte-like - type B – subependymal cells expressing glial fibrillary acidic protein (GFAP) and nestin.[107] These multipotent, type B NSC – which reside within the SVZ – are believed to generate *in vivo* at least three different populations of lineage-committed transit-amplifying progenitors [type C, type D cells and white matter progenitor cells (WMPC)] of both neuronal and glial phenotype.

Endogenous NSC for brain repair

There is compelling evidence that NSC may concur in brain repair during adult life. Notably, it has been recently shown that endogenous NSC may sustain neurogenesis and gliogenesis in response to several different injuries such as those occurring during

inflammatory, ischemic, or traumatic events.[108,109] These pathogenic events might trigger a cascade of cellular and molecular signals – possibly via the release of soluble mediators (cytokines, chemokines, metalloproteases, adhesion molecules, etc.) that are capable of supporting neurogenesis and gliogenesis which, in turn, drive brain repair. In chronic experimental autoimmune encephalomyelitis (EAE), the animal model for multiple sclerosis (MS), mitotically active progenitor cells, residing either in the SVZ of the brain or in the subependymal layer of the central canal of the spinal cord, subvert their physiological destiny – migration along the RMS to the olfactory bulb or to the lateral columns of the spinal cord – and migrate into areas of demyelination where they differentiate into glial cells.[108] In experimental models of spinal cord injury (SCI) or ischemic stroke, neurogenesis of endogenous NSC –residing close to the traumatized or ischemic regions and surviving the injury –occurs within one week after the pathogenic event. In fact, nestin-reactive proliferating progenitor cells have been found at the border of ischemic and traumatized areas while supporting post-injury neurogenesis within a zone comprised between the damaged tissue and the surrounding intact cerebral parenchyma.[109]

The accumulating evidence indicating that endogenous neurogenesis and gliogenesis occur as part of an *intrinsic* brain self-repair process during adulthood supports the ensuing idea of developing alternative therapeutic strategies for brain disorders based on the use of NSC transplants.

NSC transplantation in CNS disorders

In CNS disorders characterized by neuronal or glial loss – e.g. stroke, Parkinson's disease, MS, SCI – cell-based replacement therapies may represent a promising alternative therapeutic approach. However, there are some preliminary questions that need to be solved before envisaging any potential human applications of such therapies: (i) the ideal cell source for transplantation; (ii) the route of cell administration; (iii) the differentiation and persistence of NSC in the targeted tissue. Furthermore, functional and long-lasting integration of transplanted cells into the host tissue has to be achieved.

The source of cells

The *gold standard* cell for replacement therapies must, in essence, be plastic. SC meet this criterion since they are intrinsically able to adapt their specification fate to different environmental needs. Both ES cells and adult NSC could be ideal cell sources for cell replacement-based therapies in CNS disorders. Embryonic neural cells, although representing a promising source of NSC, have not been consistently used so far for transplantation purposes.

ES cells

ES cells can be induced to differentiate *in vitro* into almost all cell types of the body including neural cells; these can be obtained by growing the ES cells in the presence of trophic factors such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF)-2.[110] However, while totipotent ES cells have been used for transplants there are no consistent data on the use of ES-derived lineage restricted neural cells. To partially overcome such limitation, protocols have recently been developed to generate *in vitro* high numbers of cell type-specific neural precursors (e.g. oligodendroglial lineage cells, dopaminergic neurons) from ES cells.[111]

Adult NSC

NSC can be isolated from adult human brains and can be expanded and maintained safely in a chemically defined medium for years thus supporting the concept that these cells might represent a renewable source of uncommitted NSC which could be used for transplantation procedures. These cells show: (i) growth factor (GF)-dependent proliferation and a stable growth rate; (ii) capacity for self-renewal; (iii) multipotentiality; (iv) functional plasticity either over serial *in vitro* passaging or after several freezing-thawing cycles. Adult NSC plasticity and functional flexibility can be modulated *in vitro* by exposure to different growth factors. LIF, brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), neurotrophin (NT)-3, and NT-4 drive NSC to a neuronal fate (up to 40-60% of cells in culture), whereas bone morphogenetic proteins (BMPs), CNTF and LIF increase the number of NSC-derived astrocytes.[112]

In vivo experiments aimed at repairing injured CNS by transplanting multipotent NSC have shown that these cells might survive transplantation procedures within the host CNS, and can display notable migratory properties from the site of grafting and maintain their multipotency. While NSC, transplanted either intraparenchymally or intrathecally into healthy rodents, show precise pathways of tissue invasion and neuronal (i.e., dopaminergic) differentiation,[113,114] there are data indicating that this differentiation pattern changes when NSC are transplanted into rodents suffering from experimental CNS diseases. In experimental autoimmune, chemical or traumatic CNS demyelination, NSC – transplanted intraparenchymally, intracerebroventricularly (i.c.) or intravenously (i.v.) – show the ability to selectively reach the areas of tissue damage, to differentiate into axon-ensheathing oligodendrocytes and to promote functional recovery.[115] Accordingly, site-specific dopaminergic neuronal differentiation has been obtained by intrastriatal transplantation of undifferentiated syngenic or xenogenic (human) NSC in rats affected by experimental parkin-

sonism.[116] More recently, i.v.-injected human adult NSC have been efficacious in promoting functional recovery in rats affected by experimental intracerebral hemorrhage or transient cerebral ischemia via terminal differentiation, within the host brain, into neurons (10%) and astrocytes (75%).[117,118] Notably, NSC transplants – both in healthy and diseased rodents –did not induce tumor formation in immunodeficient mice, thus strongly suggesting that the *in vivo* tumorigenic potential of such a potent cell source is minimal.

BM-derived SC

BM-SC have been recently shown to be able to differentiate into neural cells when transplanted into rodents or humans.[119,120] The most challenging example of the contribution of these cells to the cytoarchitecture of the brain comes from a recent report showing Y-chromosomes in cerebellar Purkinje neurons of women who had received BM transplants from male donors.[121] Along with this evidence of BM-SC plasticity, there are other reports that collectively suggest that these cells could contribute to generating new neurons in the adult brain by means of (i) transdifferentiation (direct conversion of transplanted cells into neurons)[119,120] and/or (ii) cell fusion (assimilation of transplanted cells or their progeny into existing neurons and formation of heterokaryons).[122] The demonstration that BM-SC and NSC are developmentally plastic - although this is still a matter of debate[123,124] - has encouraged many attempts aimed at using BM-SC for brain repair. Animal models of focal demyelination, ischemic stroke, amyotrophic lateral sclerosis, and spinal cord injury have been treated with BMSC.[125-127] These studies showed that differentiation of BM-SC into brain cells can be obtained also in experimental models of brain disorders. However, only a few of these studies consistently showed that BM-SC differentiated into brain cells may induce functional repair of the brain. In rats with a demyelinated lesion of the spinal cord, BM-SC from GFP-expressing mice (immunoreactive for collagen type I, fibronectin, and CD44) produced remyelination and improvement of axonal conduction velocity when transplanted i.v. or by direct microinjection into the demyelinated spinal cord of immunosuppressed rats.[126] Donor BM-SC obtained from transgenic mice constitutively expressing β-galactosidase (transcriptionally regulated by an endothelial-specific promoter Tie2) were found, by perfusion-weighted magnetic resonance imaging (MRI), in sites of neovascularization where cerebral blood flow (CBF) was increased.[127] The clinical use of BM-SC in other experimental CNS disorders, such as amyotrophic lateral sclerosis and spinal cord injury, is too preliminary to draw any meaningful conclusion on the reparative potential of these cells.[128]

The route of cell administration

The route of cell administration is another key issue in NSC transplantation procedures for CNS diseases. On the one hand, the anatomo-pathological features of focal CNS disorders, such as Parkinson's disease or SCI, would suggest that direct intralesional cell transplantation might facilitate tissue regeneration of dopaminergic neurons of the substantia nigra or of naked axons within a specific segment of the spinal cord, respectively. On the other hand, the mutifocality of certain CNS disorders such as amyotrophic lateral sclerosis, MS, or Alzheimer's disease, limits *per se* NSC-based therapies. However, some recent experiments have shown that, at least, in multifocal inflammatory brain disorders these limitations can be overcome by injecting therapeutic stem cells (e.g. BMSC, MSC, NSC) into the blood stream or into the cerebrospinal fluid compartment. Specific homing can be explained, at least in part, by the constitutive expression by NSC of a wide array of inflammatory molecules such as adhesion molecules (integrins, selectins, immunoglobulins, etc.), chemokines, cytokines and chemokine receptors.[129]

Differentiation and persistence of NSC in the targeted tissue

Although very little is known about the mechanisms instructing the terminal differentiation of NSC *in vivo*, there is strong evidence that the local environment might dictate the fate of transplanted uncommitted NSC. In this respect, undifferentiated multipotent NSC or even totipotent ES cells, transplanted in different experimental neurological conditions, have shown considerable capacity to restrict their fate in response to tissue-specific cues and to replace non-functioning neural cells of different lineages. Totipotent ES cells display a preferential terminal differentiation into either myelinating oligodendrocytes or TH^+ neurons when transplanted into rodents affected by experimental acute SCI or 6-OH-DA-induced parkinsonism, respectively.[130] Even more efficiently, multipotent growth factor-responsive NSC show neuronal- or glial-restricted fate when transplanted in animal models of neuronal (e.g. Parkinson's disease, stroke) or myelin dysfunction (e.g. EAE, SCI),[113,115,131-133] respectively. Thus, the local environment may dictate the fate of transplanted NSC. However, transplanted NSC might exert their therapeutic effect not only by differentiating into lineage-restricted daughter cells and by functionally integrating into the host tissue. It has recently been shown that upon transplantation – independently of the characteristics of the injured area of CNS into which the NSC have been transplanted – NSC might remain in an undifferentiated state (e.g. lack differentiation antigens, round-shaped morphology, perivascular localization) but continue to release neurotroph-

ic growth factors (FGF-2, BDNF, GDNF, etc.).[115] This evidence suggests that NSC might repair brain damage also by remaining in their undifferentiated state and acting as bystander regulators of neuronal and/or oligodendrocytic rescue via the constitutive or induced release of neurotrophic molecules.

Epithelial SC and regenerative medicine

Epidermal and limbal SC

Human epidermis consists of a stratified squamous epithelium composed of keratinocytes organized in distinct cellular layers. Basal keratinocytes that become committed to terminal differentiation stop dividing, migrate upwards and initiate the process of keratinization. This leads to the sequential formation of the stratum spinosum, stratum granulosum and stratum corneum, the last being composed of anucleate corneocytes continuously shed into the environment. For each corneocyte shed from the epidermal surface, a basal keratinocyte must undergo a round of cell division to replace it.

The surface of the human eye is covered with highly specialized conjunctival and limbal-corneal epithelia, which are formed by two genotypically different keratinocytes.[134] The corneal epithelium is a transparent, flat, stratified squamous epithelium with a cuboid basal layer lying on the avascular corneal stroma by Bowman's layer. The narrow transitional zone between the cornea and the bulbar conjunctiva is referred to as the limbus. The limbal epithelium consists of several layers of keratinocytes organized into well developed rete ridges, devoid of goblet cells and populated by Langerhans' cells and melanocytes.

Epidermal epithelium is replaced approximately monthly whereas corneal epithelium is replaced about every year. To accomplish their self-renewal process, these epithelia rely on the presence of stem and transient amplifying (TA) cells, the latter defined as non-stem daughters of SC that are able to divide only a few times before the onset of terminal differentiation.[135-136] Separate populations of SC have been suggested to exist in interfollicular epidermis, hair follicles and sebaceous glands, at least under steady-state conditions.[136-142] However, the bulge-containing region of the hair follicle is thought to represent the main reservoir of skin keratinocyte SC.[136,140] Human corneal SC are located exclusively in the basal layer of the limbal epithelium.[138,141] Primary cultures of human keratinocytes can be used to identify and characterize epithelial SC. Three types of keratinocytes with different proliferative capacities have been identified in human epidermis, hair follicles, limbal and conjunctival epithelia, and are referred to as holoclones, meroclones and paraclones.[137,138,142,143]

The holoclone, considered the keratinocyte SC.[137] The holoclone is identical to the multipotent SC located in the bulge of the hair follicle, is located in the limbal but not in the corneal epithelium, generates a mature epithelium *in vivo*, has telomerase activity, long telomeres and an astonishing proliferative potential,[137,138,142,143] which are all hallmarks of SC. A single holoclone can produce as many as 10^{45} keratinocytes before senescence, which occurs after more than 180 cell divisions in culture and dozens of cell passages. A human being contains 8×10^{10} epidermal keratinocytes scattered on approximately 2 m^2 of body surface. Thus, a single holoclone can generate enough epithelial cells to cover 1.25×10^{34} human beings or an area equivalent to 2.1×10^{28} Km^2.

The paraclone is a TA cell, which is committed to a maximum of 15 cell divisions and generates aborted colonies containing only terminally differentiated cells.[137,138] The meroclone is an intermediate type of cell and is probably a reservoir of TA cells.

Cultured epithelial SC and regenerative medicine

Keratinocyte SC are currently used to permanently restore many severely damaged epithelial surfaces, such as epidermis, cornea and mucosal membranes.[144–149]

Human epidermal keratinocytes propagated *in vitro* generate sheets of stratified epithelium which maintains the characteristics of authentic epidermis, including functional melanocytes physiologically organized in the basal layer. Autologous cultured keratinocytes have been used worldwide to regenerate the epidermis in thousands of burn victims.[144,150] Human epidermis is renewed monthly. Permanent epidermal regeneration – over 20 years of follow-up and hence over 200 renewing cycles – has been achieved in these patients. Thus, this technology has been proven to be life-saving. A mature epidermis regenerates within a week after grafting.[144,145] Hemidesmosomes and basal lamina reform conjointly and regenerate a functional dermal-epidermal junction within the first month after transplantation, although full maturation of anchoring fibrils requires approximately one year. A mild hyperkeratosis is usually present at 4-8 months after grafting and disappears afterwards. The dermal-epidermal junction is usually flattened during the initial semester. Shallow but defined rete ridges appear after 8-12 months and their depth progressively increases. The regenerated epidermis contains a normal population of Langerhans' cells and melanocytes, the latter forming an epidermal melanin unit already at 4 months after grafting. Normal histologic features are maintained for years after transplantation. Pathologic, dysplastic or pre-malignant changes are not observed.

Epidermal grafts for burn victims are usually prepared from full-thickness skin biopsies taken from hairy body sites in order to maximize the number of SC used for graft preparation. Therefore, in most

patients, it is not possible to establish whether regenerated epidermis originated from interfollicular or hair follicle SC.

Ocular chemical burns are characterized by a depletion of limbal cells thereby leading to corneal re-epithelialization by bulbar conjunctival cells.[151] This abnormal wound healing process induces neo-vascularization, chronic inflammation and stromal scarring. Total limbal SC deficiency eventually leads to corneal opacification and visual loss. Allogeneic corneal grafts, aimed at replacing the corneal stroma, are not successful in these patients, unless the limbal SC population has been restored by limbal grafts taken from the uninjured eye.[152] It has been shown that cultures of autologous limbal SC, isolated from a tiny (1-2 mm^2) limbal biopsy taken from the uninjured eye, permanently (over 5 years follow-up to date) restore corneal integrity and function, that is normal visual acuity, in patients with unilateral limbal stem cell deficiency unresponsive to conventional surgical therapy, such as penetrating or lamellar keratoplasty.[146,148] The corneal epithelium and visual acuity can also be restored in blind patients presenting with bilateral limbal SC deficiency unresponsive to multiple keratoplasty.[152,153] These patients can be treated with allogeneic cultured limbal cells. The persistence of donor cells has been formally proven at the last available (3 years) follow-up.

However, restoration of bilateral SC deficiency by means of cultured allogeneic limbal SC requires immunosuppressive therapy. Further studies are required to investigate whether SC isolated from other stratified epithelia have enough plasticity to generate an authentic corneal epithelium. Indeed, if autologous epidermal or oral mucosa SC could be reprogrammed to produce limbal SC, corneal epithelium could be regenerated in patients with bilateral limbal SC deficiency without the need for immunosuppression. Urethral epithelium has been generated by cultured urethral SC in patients with posterior hypospadias, which is characterized by the congenital absence of urethral epithelium in the entire penis.[149] Palate-derived epithelial cells have been used to regenerate palate epithelium and gingival mucosa.[154]

The sum of clinical data accumulated during the last 2 decades proves that cultured epithelial SC can regenerate a functional tissue when transplanted back into patients. Collectively, the permanent epidermal regeneration achieved in burned patients by means of keratinocytes cultivated from glabrous skin, the maintenance of palm/sole-specific histological and biochemical features in the regenerated epidermis and corneal regeneration by means of allogeneic cultured limbal cells provide compelling evidence that (i) keratinocyte SC are maintained in culture, even if they are released from the *in vivo* control mechanism designed to keep them in a slow-cycling state; (ii) cultured keratinocyte SC take and regenerate a renewing epithelium *in vivo*; (iii) keratinocytes endowed with authentic SC properties are also located in the interfollicular epidermis. These data add strength to the notion that the bulge-containing region of the hair follicle is likely to represent the main reservoir but not the only residence of keratinocyte SC, and that bulge-derived SC are likely to play a role in regenerating epidermis only in emergency situations.[136] For instance, it is common knowledge that partial-thickness burns, which are characterized by total epidermal destruction but preservation of skin appendages, can be healed by keratinocytes migrating from hair follicles. It is also likely that SC migrating from the bulge can restore the population of SC normally present in the epidermal basal layer.

Cartilage and bone repair

Mesenchymal stem/progenitor cells

Friedenstein *et al*. were the first to identify a cell population with a strong osteogenic potential in a single cell suspension from adult BM.[155]

Osteogenic cells were similarly derived from the thymus and the spleen of guinea pigs and rabbits. Following this pioneering work, many research groups have more recently isolated mesenchymal stem/progenitor cells from adult tissues. These cells are able to undergo differentiation pathways other than those they were initially determined to follow. Several groups have isolated pluripotent stem/progenitor cells from the BM stroma. BM stroma is composed of a heterogeneous variety of cell types among which BM stromal cells are the essential elements of the hematopoietic microenvironment, via direct cell-cell contact and by supplying soluble mediators.[156]

Recent studies demonstrated the pluripotency of BM stromal cells showing that they can differentiate into ectodermal and endodermal cells such as astrocytes, oligodendrocytes, neurons, hepatocytes (*see above and Table 4*). Nevertheless, it is questionable that BM stromal cells are indeed a homogeneous population of adult SC. On the contrary, several pieces of evidence indicate that BM-SC are a very heterogeneous population comprising cells at different stages of commitment and that cells with stem/early progenitor characteristics, if present, are only a subpopulation of the whole cell population. Plated at low density, BM stromal cells form distinct and heterogeneous colonies derived from a single cell (colony-forming units-fibroblastic: CFU-F). Colonies are different in shape and size, reflecting differences in the proliferation and growth rate of the CFU-F. After some time in culture, phenotypic differences are more evident. Some CFU-F are tri-potential in that they differentiate *in vitro* into osteoblasts, chondrocytes and adipocytes, others are bi-potential, differentiating only towards a chondro-osteogenic phenotype, and a third group includes only osteogenic clones.[156] During culture, tri-potential clones

first lose adipogenic differentiation, then chondrogenic and lastly osteogenic potential. The heterogeneity of the BM stromal cell clones is also observed *in vivo*, since only 60% of clones are osteogenic.[158] As discussed above, Jiang *et al*.[17] have also isolated from the BM a cell population, MAPc, with true SC properties. Pluripotent cells, with properties similar to those from BM, have also been isolated from human adipose tissue.[159] Capillary pericytes are probably an additional source of cells with stem/progenitor properties. It has been suggested that marrow pericytes and marrow stromal cells are the same cell population.[160] The pericyte is a cell of mesenchymal origin with long cellular processes surrounding capillaries. Pericytes have been isolated from capillaries of different tissues, including cerebral cortex, peripheral nerves, retina, skeletal muscle and the lungs. When implanted in diffusion chambers *in vivo*, some of these pericytes spontaneously form bone and cartilage.[161] Vascular smooth muscle cells produce bone-specific matrix proteins such as osteopontin, osteonectin, and matrix gla protein.[162] Osteoprogenitors have been isolated from peripheral blood, although the efficiency of these progenitors is very low, [163] and from the skeletal muscle.[164]

Engineering cartilage and bone

Implanting stem/progenitor cells in a suitable scaffold versus implanting of a preformed tissue

Taking advantage of cells with chondro-osteogenic potential, two main approaches have been proposed for the repair of traumatic lesions of cartilage and bone: (i) implanting undifferentiated pluripotent cells in a carrier scaffold and (ii) implanting a tissue preformed *in vitro*.The first approach relies on the fact that pluripotent cells are activated toward a specific differentiation pathway in the appropriate environment. According to the second strategy, undifferentiated pluripotent cells are associated with a suitable scaffold and cultured *in vitro* within a bioreactor under conditions favoring the formation of transplantable tissue. In both cases the choice of the optimal cell type and the choice of the most suitable scaffold are critical for the success of the therapy.

Dedifferentiated chondrocytes

Human articular chondrocytes derived from articular cartilage biopsies and grown as a monolayer dedifferentiate with culture passages and lose their chondrogenic phenotype. It has been suggested that dedifferentiated chondrocytes are cells that have regressed to a more primitive stage thus being able to redirect their differentiation towards a different phenotype and therefore, having the same properties as pluripotent MSC. Nevertheless, cultured dedifferentiated chondrocytes have only a limited proliferative potential. The number of cell divisions they can undergo decreases with the increase of the donor's age.[165] In addition, within a few culture passages, dedifferentiated chondrocytes lose their ability to redifferentiate upon *in vitro* transfer into a 3-dimensional environment[166] and upon subcutaneous implantation into an immunodeficient mouse. The presence of growth factors, such as basic fibroblast growth factor (FGF-2) and transforming growth factor β1 (TGFβ1) in the culture increases their proliferation rate and maintains their redifferentiation potential longer. Human articular chondrocytes grow well in medium supplemented with serum, but in order to be able to redifferentiate both *in vitro* and *in vivo* they need to be rescued by strong chondrogenic inducers (TGFβ1). In contrast, chondrogenic commitment is maintained when chondrocytes are cultured in a defined medium supplement, in which serum is substituted by specific growth factors (in particular FGF-2).

Mesenchymal stem/progenitor cells

Cells with chondro-osteogenic potential have been isolated from several tissues, including periosteum, BM, spleen, thymus, skeletal muscle, adipose tissue, skin and retina.[17,65,159,161,164] However, for several reasons (limited or hardly accessible tissue source, low frequency of stem/progenitor cells in the cell population, limited information on the specific cell system, etc.), BM remains, at the moment, the most widely used cell system in preclinical and clinical studies on both bone and cartilage regeneration and repair. The collection of a limited amount of BM cells is an easy and relatively safe procedure. BM stromal cells can be expanded in culture, making it possible to engineer transplantable constructs made by these cells associated with appropriate scaffolds.

BM stromal cells are responsible for the maintenance of bone turnover throughout life and can be regarded as mesenchymal progenitor/precursor cells derived from adult stem cells. Cultured BM stromal cells can be stimulated to differentiate into cells of tissues of mesodermal origin such as bone, cartilage, muscle, marrow stroma, tendon, fat and a variety of other connective tissues[167] and, under certain conditions, also into cell types derived from ectoderm and endoderm (*see above*). When implanted into immunodeficient mice, BM stromal cells combined with mineralized three-dimensional scaffolds form a highly vascularized primary bone tissue.

In vitro culture: a critical step for mesenchymal stem/progenitor cells

The use of BM stromal cells for the reconstruction of bone segmental defects requires extensive *in vitro* expansion of the cell population maintaining a high regenerative potential. The properties of BM stromal cells are profoundly influenced by the microenvironmental conditions, including culture conditions. Upon

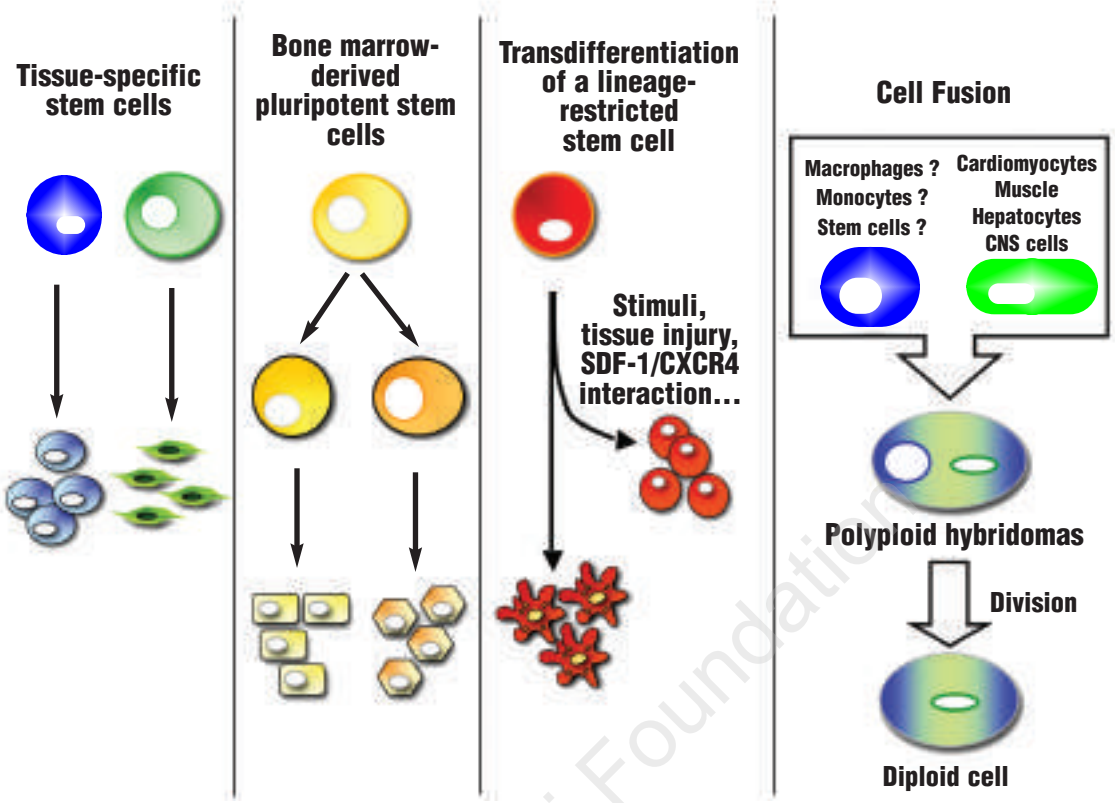

Figure 2. Hypotetical models of adult tissue regeneration and stem cell developmental plasticity.

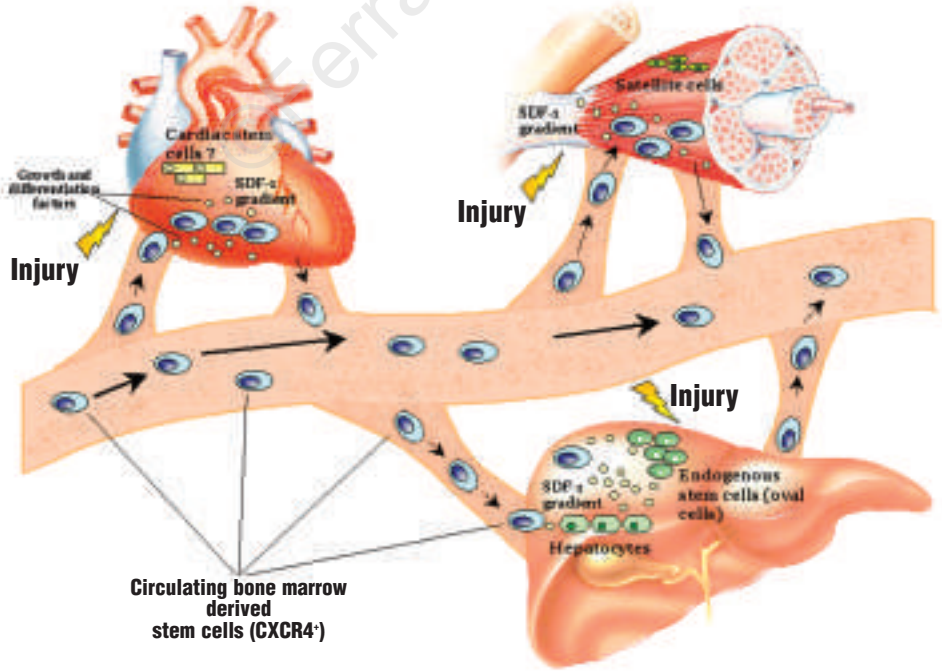

Figure 3. Bone marrow-derived stem cells are recruited to the site of tissue damage via SDF-1/CXCR4 interaction

in vitro expansion, the bone-forming efficiency of *in vivo* transplanted BM stromal cells is significantly reduced.[157] Culture conditions that allow BM stromal cell expansion without loss of differentiation potential are difficult to establish. Requirements for *in vitro* expansion of BM stromal cells have been investigated.[168-172]

The CFU-F derived from BM cells undergo clonal osteogenic, chondrogenic and adipogenic differentiation. When clones were isolated and expanded in the presence of FGF2, the frequency of clones able to differentiate into the osteogenic, chondrogenic and adipogenic lineages (*tripotential* clones) increased. This effect was associated with the cultured cells having longer telomeres. The observed increase in telomere length is due to the selection of a subset of progenitor cells. In the best culture conditions, these cells have an extended life-span of about 70 doublings and they retain their differentiation potential for over 50 doublings.

Culturing BM-SC in the presence of FGF2 is a critical step for osteogenic development also *in vivo*. At 4 weeks, bone was observed only in implants loaded with FGF2-expanded BMSC; at 8 weeks bone was more than 12% of the total tissue formed in the case of implants loaded with cells expanded in the presence of FGF2 while in the control implants, loaded with cells expanded in the absence of FGF2, bone formation accounted for only 0.5% of the tissue.[172]

The above data support the concept that BM-SC are not a homogeneous population of MSC. On the contrary, they are a heterogeneous population of mesenchymal progenitor/precursor cells. Adding FGF2 to the culture, maintains cells in a more immature state and allows the *in vitro* expansion of osteo-progenitors, possibly by selecting a specific cell subset. FGF2 is not sufficient by itself to prevent progressive cell senescence but significatintly increases the frequency of multipotential clones and allows a large number of cells, with preserved differentiation potential, to be obtained for mesenchymal tissue repair.

Choice of suitable scaffolds

Several scaffolds have been developed and used for tissue engineering. The ideal scaffold is non-immunogenic, non-toxic, biocompatible, biodegradable and easy to manufacture. Scaffolds should permit easy diffusion of nutrients and cellular waste products. At the same time, they should provide initial good mechanical support for cells and extracellular matrix during the repair process. The degradation rate of the scaffold should be tuned to the rate of production of extracellular matrix by the cells.

Scaffolds currently used for cartilage repair are made of both naturally occurring polymers and synthetic polymers. Collagen (in most cases type I collagen derived from porcine and bovine skin), fibrin, (currently used in the clinic as fibrin glue), and hyaluronic acid (often after partial esterification) are the most frequently used natural polymers. Examples of synthetic polymers are polylactic acid and polyglycolic acid (homo- or heteropolymers), and polycaprolactone. All these polymers can be shaped into membrane films, threads, sponges and hydrogels.

Porous bioceramics made of hydroxy-apatite (HA), tri-calcium phosphate (TCP) or a mixture of the two are the most widely used scaffolds for bone repair. A relatively higher concentration of TCP in the bioceramic usually results in higher resorbability. These ceramic scaffolds have osteoconducive properties and can *integrate* with the patient's bone tissue.

Alternative mineralized scaffolds are derived from cadaveric or animal bones, and from coral exoskeleton. Bioreactors could improve the production of transplantable engineered tissues and in particular of engineered cartilage by providing an efficient and spatially uniform cell seeding in three-dimensional scaffolds and by allowing better nutrient diffusion. Furthermore, bioreactors can expose forming engineered cartilage to specific physical stimuli.

Preclinical and clinical studies

Cartilage repair

Autologous chondrocyte implantation (ACI)

Chondrocytes are isolated from a biopsy of healthy articular cartilage from a low weight-bearing area of joint and expanded in monolayer culture. Chondrocytes are reinjected under an autologous periosteal flap, sutured and/or glued onto the cartilage defect[173] and good to excellent results are obtained in more than 90% of cartilage lesions.[174]

Limitations of the classical ACI procedure are related to chondrocyte culture conditions and graft fixation methods, especially for osteochondral defects. As possibe solutions to overcome some of these problems, cell delivery systems based on collagen membranes or three-dimensional hyaluronic acid scaffolds have been proposed.[175]

Engineering cartilage

The use of *in vitro* engineered cartilage for treatment of cartilage lesions is still at a preclinical stage. To test the chondrogenic potential of human cells and their interactions with natural and synthetic scaffolds, constructs can be implanted subcutaneously into immunodeficient mice. Nevertheless, in this animal model, the results can be significantly biased by the absence of biomechanical forces acting on the forming cartilage.

Several experiments have been performed in rabbit models to test the efficacy of different scaffolds, but graft survival and engineered cartilage remodeling

under loading are under investigation in weight-bearing large animal models (e.g., sheeps, goats, dogs, and horses).

Repair of large bone defects

Systemic injection of osteogenic cells

Conflicting data concerning BM stromal cell engraftment after systemic injection have been reported in the literature. Following intravenous infusion, SC are capable of homing not only to the BM of immunodeficient[176] or irradiated mice[177] and irradiated baboons,[178] but also to multiple sites, such as bone, cartilage, lungs and spleen.

In humans, allogeneic BM transplantation has been used to treat 3 children with severe osteogenesis imperfecta.[179,180] Three months after transplantation, total body bone mineral content was increased and new dense bone formation was observed in trabecular bone. The clinical conditions were, perhaps, improved by engraftment of functional mesenchymal progenitor cells. BM stromal cell engraftment has been clearly shown in sheep fetuses. Co-transplantation of human stromal cells improved the long-term engraftment of HSC, whereas stromal cells transplanted alone remained functional in the hematopoietic microenvironment[181] and persisted in multiple tissues undergoing site-specific differentiation for more than 1 year after the transplant.[182]

Engineering bone

Based on the encouraging results obtained with rodents and large animal models, clinical trials were initiated in humans with large (4 to 7 cm) bone defects, in whom traditional therapeutic approaches had proven unsuccessful.[183] Whole BM was harvested from each patient, *ex vivo* expanded BM stromal cells were loaded on a macroporous hydroxyapatite scaffold of the shape and size of the missing bone segment and the scaffold implanted into the bone defect. External fixation was provided for initial stability. All patients recovered limb function by between 6 and 12 months. The expected recovery time after a traditional approach would have been much longer, under the most favorable conditions and in the absence of complications.

Results so far obtained in cartilage and bone tissue engineering are very encouraging, but still not optimal. Additional experimental and clinical work is needed before this therapeutic approach can be routinely used in clinical practice. Questions requiring answers and problems awaiting solutions regard the optimal origin of cells, the availability of well-defined serum-free media for SC culture, the possibility of using allogeneic cells and the development of a new generation of reabsorbable scaffolds and more advanced bioreactors.

Conclusions and perspectives

Adult, tissue-specific SC have proven to be a powerful therapeutic tool for regenerative medicine. In particular, BM and circulating HSC are routinely used to restore altered hematopoiesis. However, although the proof of principle that BM-SC contribute to non-hematopoietic tissues has been provided, numerous studies have shown that the phenomenon of SC developmental plasticity is rare and its frequency may not be, at present, therapeutically relevant. The recruitment of BM-SC to other tissues seems to be injury-dependent and the key regulators of *transdifferentiation* are still unknown. Taken together, the data reviewed here indicate that the physiological relevance of SC plasticity may be negligible. However, it is possible that adult SC could eventually be used for clinical programs of regenerative medicine. To achieve this goal, basic scientists need to better characterize the cell population responsible for the generation of mature cells of different tissues [SC (?) Mature precursors (?)]. We also need a better understanding of the molecular mechanisms regulating the balance between SC self-renewal/differentiation and how to induce more efficient production of mature tissue cells. Identification of the tissue-specific and injury-related signals that regulate homing, engraftment and expansion of BM-SC at the site of tissue damage as well as an understanding of the mechanisms inducing fusion or plasticity should also help to increase the therapeutic potential of adult SC (Figure 2). Moreover, recent data indicate that, along with tissue-specific SC, there may be a rare pluripotent SC population, which circulates in the PB at low frequency and can be recruited at the site of tissue damage for tissue repair (Figure 3).

Of note, the controversy between *cell fusion* vs *transdifferentiation* has been further fuelled by very recent data showing differentiation of HSC into liver cells[59] and NSC into endothelial cells,[124] *in vitro*, without cell fusion. However, cell fusion may be considered a mechanism of cell plasticity, although not necessarily involving SC.[184,185] In addition, from a therapeutic point of view, as long as the function of the damaged tissue is restored it may not be crucial that true transdifferentiation occurs, as demonstrated in the FAH model of liver injury. Additional strategies to improve the therapeutic potential may be *ex vivo* culture of BM-SC to induce their initial commitment to customized tissue cells, genetic manipulation of stem/progenitor cells to induce their proliferative advantage over resident cells, increasing the number of SC available for tissue repair by the use of hematopoietic cytokines (e.g. G-CSF, SCF) to mobilize HSC or the post-transplant use of cytokines such as HGF in the case of liver tissue damage. Finally, the very concept of SC as a definite *entity*

is under discussion.[186] Data discussed in this review indicate that the *function* of SC can sometimes be acquired by more mature progenitor/precursor cells under appropriate microenvironmental *stimuli*.

This finding supports the view that cycling progenitors, as opposed to quiescent SC, may activate different genetic programs leading to transdifferentiation (Figure 1).

Although these issues need to be properly addressed, several clinical trials have already been completed and many more are underway to test the concept of adult SC developmental plasticity.

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