Stem Cell and Regenerative Medicine

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Abstract: Stem cells have been identified and isolated in many adult tissues. They exhibit a great plasticity and the ability to give rise to differentiated cells of several lineages. The possibility of transplantation of these stem cells into an adult to develop, integrate and rebuild destroyed tissues or organs has encouraged the study of the mechanisms of the differentiation of stem cells. These cells are nowadays being called a panacea in numerous diseases and, although their functional role is not well known, they are present in several areas of the human therapy, increasing the clinical applications. They represent the future of the transplant in medicine, and open, moreover, new perspectives in the treatment of diseases, as it is the case of the regenerative medicine. Here we review the current literature examining several aspect of medical therapy such as the applicability of experimental models to clinical practice.

Keywords: stem cells, neural stem cells, neurogenesis, dental stem cells, odontogenesis, regenerative medicine.

1. INTRODUCTION

The stem cells, firstly suggested by Danchakoff [1] and by Sabin [2] and Maximow [3] may be defined as cells capable of proliferation, self-maintenance, and the production of differentiated, functional progeny that are characteristic of the organ from which they were derived [4-6].

Stem Cells can be classified according to:

1. Potential for differentiation into totipotent, pluripotent, multipotent, and unipotent cells.
2. The tissue of origin for embryonic or adult stem cells.
3. Their capacity for tissue re-population *In vivo* in short, medium or long time regeneration.

In addition to the different classifications of the stem cell, this cell type also generates great interest in relation to the different models of cellular differentiation; these include the mother-daughter classic cell model, multiple lineages restricted stem cells, transdifferentiation of lineage restricted stem cells; and for adult somatic stem cells: dedifferentiation of mature cells followed by redifferentiation.

The possibility of differentiating or redifferentiation stem cells for their use in the treatment of diverse degenerative diseases gives us a glimpse of their extraordinary prospects. Nevertheless and even though technical problems are gradually being solved, the status of stem cells is being reviewed and although great progress is being made in the different fields as odontogenesis, neurogenesis or therapeutic clonation regarding the future of stem cells, there are still many aspects that need to be clarified.

2. STEM CELLS

Patients suffering from diseased and injured organs may be treated with transplanted organs; however, there is a severe shortage of donor organs that is worsening yearly, given the ageing populations. One of the limitations of applying cell- based regenerative medicine techniques to organ replacement is the difficulty of growing specific cell types in large quantities. In certain situations, stem cells on account of their proliferative capacity combined with the ability to become specialized are envisioned as being an alternative a source of cells from which for the desired tissue can be derived. Many patients with extensive end-stage organ failure, a tissue biopsy may not yield enough normal cells for expansion ant transplantation [7].

Stem cells can be used to restore tissue function as integrated in the target tissue, the selected cells can be expanded in culture, induced to differentiate with appropriate combinations of signal proteins and growth factors and finally transplanted *In vivo* with the use of materials from engineered tissues, biocompatible and biodegradable scaffolds, on which the single cells can organize into tissue structures that are genetically identical to that of the host [8, 9]. Another goal of regenerative medicine using stem cell as vehicles that deliver complex signals to a target tissue without actually integrating into the tissue itself [10]. The work of Mirotsou [11] reports that mesenchymal stem/ progenitor cells mediate tissue repair through paracrine mechanisms, secreted frizzled related protein Sfrp2 to modulate Wnt signalling. Cardiomyocytes treated with secreted frizzled related protein increase cellular-catenin and up-regulate expression of anti-apoptotic genes. In spite of these results that appear to reveal the paracrine effects of mesenchymal stem cells on myocardial survival and repair after ischemic injury, for the moment, research is still insufficient. The tissue trophic effects of MSCs are being elucidated y their therapeutic effect therefore is doubtful [10].

The use of adult stem cells in research and therapy is not controversial because the production of adult stem cells does not require the destruction of an embryo. Adult stem cells can be isolated from a tissue sample obtained from an adult, Fig. (**1**). They have mainly been studied in humans and model organisms such as mice and rats.

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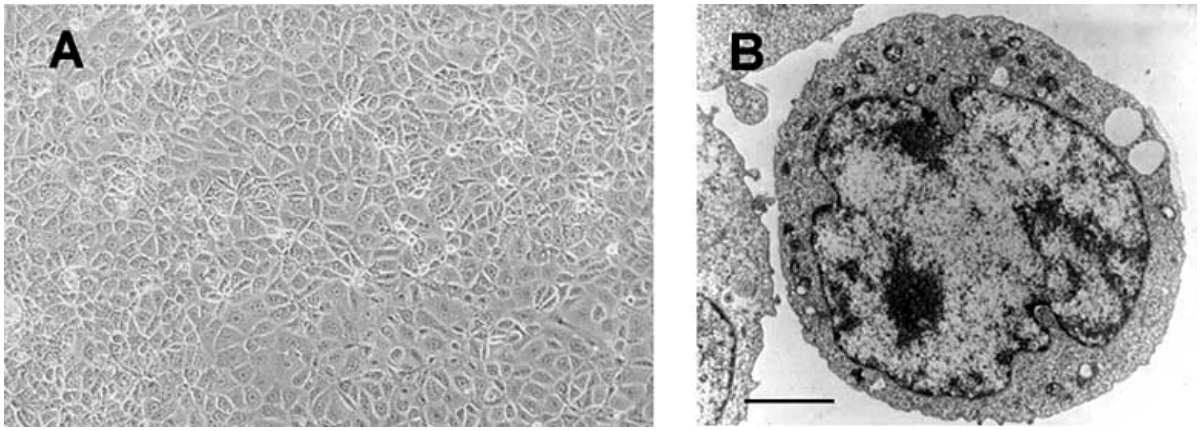

Fig. (1). Stem cells coming from an experimental teratocarcinoma of the testis of strain 129 adult mouse growing intraperitoneally. A) Cells exhibit a homogeneous appearance in a 6-day monolayer (phase-contrast microscopy, original magnification 6x). B) In electron transmission microscopy cells show a loose chromatin, evident nucleolus and cytoplasm consisting preferentially of ribosomes. (Bar: 2μ)

Multipotential stem cells are present in many tissues or organs for tissue maintenance and repair. These stem cells can give rise to a limited set of adult tissue types, blood stem cells, epidermal stem cells, neural stem cells. The ability of a stem cell of one lineage to become another lineage is called transdifferentiation or plasticity [12]. Stem cells with this property are denominated pluripotential. The multipotent stem cells are not as easy to use as pluripotential stem cells. Different types of adult stem cells are capable of transdifferentiation more than others. Pluripotential adult stem cells can be Haematopoietic Stem Cells (HSCs) when they are cultivated with a particular mixture of paracrine factors [13]. HSCs found in:

1. Bone marrow, called mesenchymal stem cells (MSCs), human MSCs, bone marrow-derived pluripotent adherent cells of mesenchymal origin can differentiate along the osteogenic, chondrogenic, adipogenic, and tendonogenic lineages [14].
2. Peripheral blood.
3. More recently, umbilical cord blood [15].

Although studies suggest efficacy with no obvious safety concerns the mechanism for this therapeutic effect is unknown. In addition, data indicate that "transdifferentiation" of circulating HSCs and/or their progeny is an extremely rare event, if it occurs at all [16]. For many there is no evidence of its occurrence, moreover they are difficult to isolate, are often fewer than out of every thousand cells in an organ. In addition, they appear to have a relatively low rate of cell division. However this has not been an impediment in certain cases of bone marrow transplantation for it to be used in the transfer of hemapoietic stem cells from one person to another, these transplants being efficacious in patients for people who are suffering from red blood cell deficiencies, leukemias and related bone/blood cancers. Also the use of stem cells to repair wounds, particularly to the skin and cornea, is now an increasingly routine part of clinical practice [17]. When multipotential adult stem cells have been successfully isolated and cultivated with adequate paracrine factors they have proved to be very useful, Carvey and colleagues [18] have succeeded in achieving neural stem cells from midbrain of adult rats that will differentiate into dopaminergic neurons that can cure the rat version of Parkinson disease. Whether multipotential stem cells can become pluripotential stem cells is controversial.

Finally, adult stem cells are rare in mature tissues and methods for expanding their numbers in cell culture have not yet been worked out, needed large numbers of cells are need for stem cell replacement therapies. Knowledge of biological mechanisms that control stem cell function *In vivo* will help us to be able to overcome the obstacles that limit cell expansion *in vitro* and for the ultimate design of stem-cell therapeutics. In this sense, it is of great interest the observation that transplanted stem cells survive and grow only in particular tissue localizations. This supported the niche concept initially theoretical, local microenvironment tissue that maintain and regulate self-renewal and differentiation of stem cells [19]. Those stem cell populations, established in 'niches', regulate the tissue generation, maintenance and repair. The interplay between stem cells and their niche creates the dynamic system necessary for sustaining tissues, as well as for exploring the conditions that promote differentiation [20-22]. The characterization of stem cell niches in mammalian tissues [23], the environmental mechanism that maintain and regulate stem cell function in their the privileged sites in specific organs [24] open the door for the solution of methodological and technical problems in the generation of stem cells in adult tissues.

3. NEURAL STEM CELLS IN THE ADULT BRAIN

Understanding of stem cells in the central nervous system (CNS) is influenced by the difficulty to study individual stem cells *in vivo* and by the extrapolation of the results obtained with different *in vitro* techniques. Moreover, differences among species must be considered. During many years it has been considered that the adult mammalian central nervous system (CNS) has not capacity for renewing because it does not contain stem cells as other tissues. In 1962 and following decades this all changed when Altman [25] gave evidence of the presence of cells with mitotic activity pointing to the existence of neural stem cells [26].

Neural Stem Cells (NSC) are self-renewing, multipotent cells that proliferate and can give rise to any cell type present in the nervous system. They undergo either a symmetric cell division, whereby two equal NSCs are born, or an asymmetric division yielding a stem cell and a progenitor (lineage-

specific precursor with a more restricted development potential) that gives rise to the main phenotypes of the nervous system (neurons, astrocytes and oligodendrocytes). These multipotent cells are relatively mitotically quiescent stem [27] and were first isolated from adult mice striatum by Reynolds and Weiss in 1992 [28]. NSCs are present in specialized zones referred to as "neurogenic niches". In the adult mammalian brain there are two germinal zones: the subventricular zone (SVZ) in the lateral ventricles (a thin layer lining the inner wall of the lateral ventricles) and the subgranular zone (SGZ) within the dentate gyrus of the hippocampus (at the interface of the hilus and the granule cell layer). Although it is a general assumption that NSCs are a homogeneous population of multipotent progenitors, recently Merkle *et al.* [29] have shown that NSCs are a restricted and diverse population of progenitors that are not readily respecified by environmental factors present in the brain. The above authors speculate over the possibility that the activity of stem cells is regionally modulated so as to regulate the production of different types of neurons.

It is hypothesized that these NSC in the adult brain derive from stem cells [28, 30-32]. Althougth the origin and identity of the NSC remains to be elucidated, multiple cell types with neural stem cell capabilities have been identified in the central nervous system, such as differentiated ependymal cells of the adult subventricular zone [33], astrocyte-like cells in the subventricular zone [34-38] and radial glia [39, 40]. Recently, Mo *et al.* [41] have detected in the human fetal brain, proliferating cells labelled with neuronal but not astrocyte markers, suggesting a further class of neuron-restricted progenitor contributing to corticogenesis.

Radial glia cells derive from the neuroepithelium [42] and they are the first glial population to be distinguished from neuroepithelial cells and are identifiable by their morphological and molecular characteristics shared with neuroepithelial cells and astrocytes. Radial glia cells are both proliferative as well as serving as migratory guides for neurons [43]. In mammalian, the radial glia are the principal stem cells of the embryonic and early postnatal mouse brain [44, 45] and give rise to adult neural stem cells in the subventricular zone [46] that express markers typical of astrocytes such as the glial fibrillary acidic protein [47], although they display functional characteristics intermediate between astrocytes and radial glia [48]. It has also been suggested there is a connection between the radial glia and the astrocytes of the SGZ [49] that remain neurogenic during adult life. Thus, the astrocytes of the SVZ and SGZ function as adult stem cells whereas parenchymal astrocytes seem to be intrinsically different to SVZ and SGZ astrocytes and do not have the capability of becoming neurogenic [50]. Signalling *via* Notch [51] and the Pax 6 transcription factor [52, 53] play a fundamental role in their differentiation.

The subventricular zone of the lateral ventricles, (Fig. **2A**), contains the largest concentration of neural stem cells in the adult mammalian brain [35, 54-56] that exhibit astrocyte characteristics (the type B cells). Type B cells are slowly dividing NSCs that give rise to type C cells (that proliferate and amplify the progeny of type B cells), and in turn give rise to type A cells (immature neuroblasts). These neuroblasts migrate to the olfactory bulb and give rise to the granule and the periglomerular cells (both are interneurons that modulate the activity of neurons projecting to the olfactory cortex). In the SGZ, Fig. (**2B**), primary progenitors with astrocytic properties are called radial astrocytes, or type-1 cells, (they are the functional equivalents of the type B cells of the SVZ) that give rise to the immature D cells, or type-2 cells (they are the functional equivalents of the type C cells of the SVZ) [37, 57, 58] which form clusters that remain among the processes of radial astrocytes. Maturing D cells remain in the hippocampus and form new granule neurons [49]. Expression of different transcriptional factors, proteins and neurotransmitters are required for specific differences in lineage specification over time [50, 59].

The presence of multipotent NSCs able to increase neurogenesis has been observed in several regions of the adult mammalian CNS of various species (including humans) such as the spinal cord and the ventricular neuroaxis, hippocampus (dentate gyrus), cortex, spinal cord and olfactory bulb [60]. The olfactory bulb, where the sense of the smell originates, and the hippocampus, involved in learning and memory, are the regions where higher numbers of new neurons are necessary. Type A cells from the SVZ migrate to the olfactory bulb in a complex network of chains that eventually merge to form the rostral migratory stream. They then differentiate into olfactory interneurons [31].

Neurogenesis is a multisequential process intricately interspersed with gliogenesis [61], where the multipotent neural cells are dividing and giving to more stem and neural progenitor cells (NPCs). These NPCs present nestin (an in-

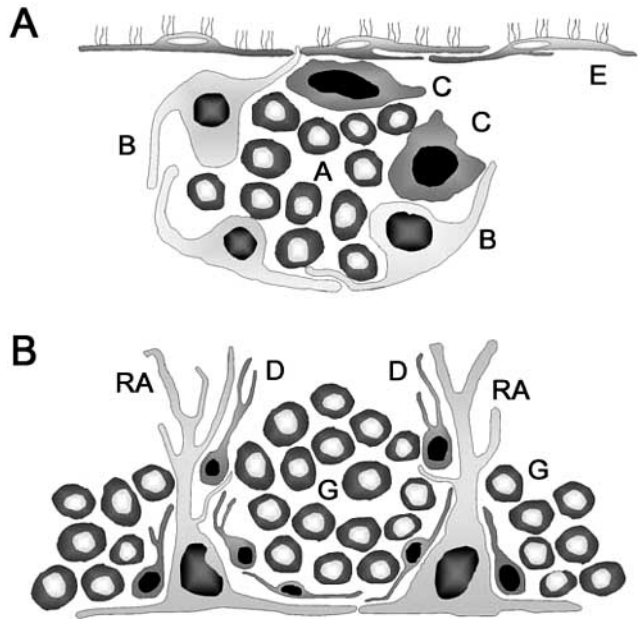

Fig. (2). Schematic architecture of neurogenic niches in the subventricular zone (SVZ) in the lateral ventricles, and in the subgranular zone (SGZ) of the hippocampus. **A**) In SVZ the ependimal cells (E) line the ventricular surface and they lie in close association with the astroglial-like cells (type B cells). Type C cells are the proliferating cells that give rise to the immature neuroblasts (type A cells). **B**) In SGZ the primary progenitors with astrocytic properties are the radial astrocytes (RA) which give rise to the proliferating D cells that remain among the processes of the RA. D cells divide and form the mature granule neurons (G cells).

termediate filament protein that is used as a marker for NSCs and NPCs) and give rise to neural and glial cells. Neurogenesis is a complex process that proceeds with input from several signalling pathways:

A) Signals that induce the proliferation of the stem cells, such as Sonic hedgehog [62, 63], Pax6 [53], basic Fibroblast Growth Factor (bFGF) [64] and Epidermal Growth Factor (EGF) [28, 65]. Vascular endothelial growth factor increases BrdUrd labelling, both in SGZ and SVZ, into neurons, astrocytes and endothelial cells *In vivo* and *in vitro* [66]. It has been suggested that Platelet-Derived Growth Factor (PDGF) affects the balance between neuroblast and oligodendrocyte precursor cell production in specific SVZ astrocytes [67, 68].

B) Signals that mark the transition between glia-like stages and neural differentiation [69], such as Notch [51], Noggin and Bone Morphogenetic Proteins (BPMs) [70-72]. Retinoic acid is required early during adult neurogenesis in the hippocampus [73]. In this respect, adult astrocytes from the hippocampus are capable of regulating neurogenesis by instructing the stem cells to adopt a neuronal fate [51, 74, 75]. Moreover, glial cells promote different aspects of neuronal differentiation by regulating transcription of different classes of genes [76].

C) Signals responsable for survival, growth and the functionality of new cells, such as the Brain-Derived Neurotrophic Factor (BDNF) [77-79] and the Insulin-like Growth Factor IGF [80]. Glial signalling also promotes neural survival [81, 82]. Wnt proteins are involved in neuroblast proliferation and commitment to a neural fate [83].

Moreover, for their maduration the new-born cells must move away from the influence of the NSCs and progenitor cells. Most of the newly formed cells die during this multisequential process [84-86], and only half of the total new cells complete all stages successfully. Finally, functional integration on a network level *in vivo* involves new neurons extending axon and dendrites to form synapses [87].

The presence of neurologic disorders along human life is unfortunately frequent. In this respect two major questions emerge:

1. What is the effect of injury on the adult NScs and neurogenic niche? and
2. How NSCs contribute to neurorepair of tissue damage

Considerable experimental evidence showing an increase in cell proliferation and neurogenesis in the forebrain SVZ of adult mammalian brain after ischemic injury has been reported [88]. The existence of neurogenesis in the neocortex after hypoxic-ischemic injury [89] is good news although its significance is a point of debate [90]. Unfortunately, most of these newly neurons have a short life.

Many neurological disorders affecting the brain are caused by a loss of neurons and glial cells. In this respect, the stimulation (by growth factor infusion or/and other manipulations) of NSCs or the transplantation of cells generated from stem cells *in vitro*, could be good therapeutical tools [91-93]. In human patients, stem based-therapies are a promising field but they are still in an early stage [94]. Thus, the control of proliferation and differentiation of these cells in an effective way to prevent tumour formation or other pathologies must be assured. For widespread use in CNS diseases there is still insufficient research available [95].

4. DENTAL STEM CELLS

The discovering of new stem cell niches in different embryonic and adult tissues makes possible the investigation of new procedures in the area of cell and tissue regeneration and therapy.

One important question concerning this matter knows where the stem cells are located in the tissues. Step by step, researchers are discovering new locations for stem cells. Nowadays, we can find more than 20 different types of stem cells locations including cardiac, brain or haematopoietic tissues, for instance. In this part of the review, we will focus on dental stem cells.

Odontogenesis as an Example of Epithelial-Mesenchymal Interactions

Organogenesis involves three important steps: initiation, morphogenesis and cell differentiation. This process starts by a cascade of cellular interactions between two embryo tissues [96]. One of the most important interactions occurs between epithelia and mesenchyme, which has been shown by using classical tissue recombination experiments [97-99]. These studies have demonstrated that the epithelial-mesenchymal interactions are reciprocal and sequential, and both components can play a role during the organogenesis depending on the organ and the stage of development. In this context, kidney, lung, mammary gland, limbs, genital tubercle, hair and teeth are different organs that develop by epithelial-mesenchymal interactions.

The beginning of morphogenesis is very similar in all these organs: the epithelium grows followed by a mesenchyme condensation around this thickening. From this stage, the development depends on the organ and the different processes are directed to the formation of specialized cells [100].

BMPs (Bone Morphogenetic Proteins, belonging to TGFβ family), SHH (Sonic Hedgehog), FGFs (Fibroblast Growth Factors), TNF (Tumour Necrosis Factors) and Wnt molecules are involved in epithelial-mesenchyme interactions, particularly during tooth development [101].

Molecular Regulation of Odontogenesis

In order to understand the different locations of stem cell niches in dental tissues we will first explain tooth development at the morphological and molecular level, summarized in Fig. (**3**).

Odontogenesis starts by the dental lamina formation followed by 4 stages: bud and cap stages for tooth morphogenesis, and early and late bell stages for odontoblast and ameloblast differentiation. In spite of specific variations in different species, these events are similar during odontogenesis in mammals.

The mouse is the most used experimental model for odontogenesis studies. In these animals, tooth development starts in E11.5 with the formation of dental lamina. This structure reveals a thickening of the oral epithelia that in-

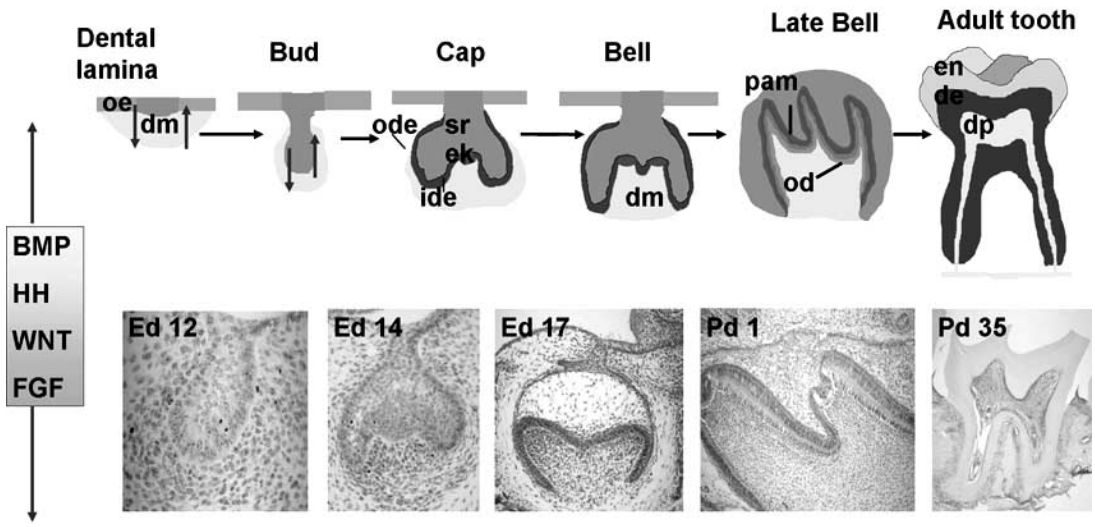

Fig. (3). Diagram of signaling molecules and growth factors in dental epithelium and mesenchyme during odontogenesis. Histological sections of mouse molar at initiation (Ed12), morphogenesis (Ed14), predifferentiation (Ed17), odontoblast and ameloblast differentiation (Pd3) in adult molar (Pd35). Ed: Embryonic day; Pd: postpartum day; oe: oral epithelium; dm: dental mesenchyme; sr: stellate reticulum; ide. Inner dental epithelium; ode: outer dental epithelium; ek: enamel knot; pam: preameloblasts; od: odontoblasts; en: enamel; de: dentin; dp: dental pulp.

vaginates into the ectomesenchyme (from the neural crest cells). In E11.5, Wnt10b, Bmp2 and SHH are expressed in the presumptive incisor and molar regions [102].

In this initial stage, the odontogenic potential is located in the epithelial cells; however, this ability is shifted to the mesenchyme at E12 [103, 104], which is induced by FGFs and BMPs from the epithelium [105]. FGFs and BMPs are antagonist molecules that stimulate and inhibit, respectively, the expression of Pax9 and Barx1. These transcription factors are very important in dental identity of molar and incisor location.

During the next stage (cap or morphogenesis stage), around E13.5-14.5 the epithelium folds and grows to surround the dental papilla mesenchyme. At this time, a signalling center is formed in the dental epithelia, called the primary enamel knot. This structure expresses different signaling molecules including SHH, BMPs, FGFs and Wnts. These signals affect epithelial and mesenchymal cells. The enamel knot regulates the patterning of tooth crown and the epithelial cervical loops. In the primary enamel knot Wnt/βCatenin pathway activates FGFs in the dental epithelia, and these growth factors activate themselves in the dental mesenchyme. In addition, mensechymal FGFs induces cell proliferation and cervical loop formation in the epithelia. It has been demonstrated that cervical loops contain stem cell niches in dental structures.

In the next stage (bell or cell differentiation stage), we can clearly distinguish the different structures of the dental epithelia: the inner dental epithelium (that develops into ameloblasts and secretes enamel), the outer dental epithelium, the stellate reticulum and the intermediate stratum. In addition, the tooth cusps become fixed and the cervical loops are still growing into the mesenchyme. On the other hand, new enamel knots are formed in the epithelia of future dental cusps. In these secondary structures, FGF4 is an activator of enamel knots, whereas BMPs and probably SHH are inhibitors and regulate the distance between the enamel knots. In the dental papilla, mesenchymal cell layer faced to the inner dental epithelium differentiates into odontoblasts and secrete dentin. Molecules such as FGFs, BMPs and TGFβ1 are responsible for odontoblast polarization and differentiation.

The final structure of the tooth is formed of dental sac cells from the mesenchyme close to the outer dental epithelium that form the dental root: periodontal ligament, cementum and alveolar bone. In addition, dentin of the root is secreted by odontoblasts facing the cervical loops, which have grown inside the mesenchyme, and finally the cervical loops are disrupted and cells separated from the structure.

Animal Models and Tooth Regeneration

Many researchers involved in tooth stem cells are focused on the study of regeneration of dental tissues using different methods that include stem cell stimulation to replace lost or damaged teeth after disease, injury or aging.

Mice have been chosen as experimental models for studying odontogenesis and tooth regeneration because human and mice show only slight differences during molar development. However, rodent incisors grow continuously. A gradient of dental epithelial cells is formed with differentiated ameloblasts and enamel, which are only found on the labial side, whereas the lingual side-lack both differentiated ameloblasts and enamel. On the contrary, odontoblasts and dentin are present both on the lingual and labial side. This asymmetry facilitates the abrasion of the incisors to keep its length relatively constant and generates a sharp tip.

Some years ago, dental epithelial stem cells were first identified in the cervical loop epithelium of the germinative ends of rodent incisors [106]. Recently, the markers of stem cells that generate enamel-producing ameloblasts have been identified [107].

Stem Cell Potential

In early embryos some single stem cells are pluripotent and have the ability to generate all the structures of the em-

bryo. These cells can give rise to more pluripotent stem cells and generate committed stem cells that lose their potential and differentiate in one specific direction. However, adult tissues still contain stem cells that contribute to renewal and regeneration of different cell populations [108, 109].

In teeth, several populations of dental stem cells have been identified [110]. Since teeth are non vital and one of the most accessible organs to obtain mesenchymal stem cells, the properties of these cells can provide a population of stem cells for experimental and therapeutic purposes [111, 112].

Dental Pulp Stem Cells

Tertiary or reparative dentin is the natural way by which teeth partially repair dentin after caries and injury that affect dental pulp. This natural mechanism shows how dental pulp cells have the ability to differentiate into odontoblast-like cells and secrete a new dentin under certain conditions. This differentiation can also be experimentally induced *in vitro* after exposition of dental mensenchymal cells to specific growth factors [113, 114]. These results clearly suggest that undifferentiated mesenchymal cells are present in the pulp and are capable of differentiating into odontoblastic cells and secreting dentin, when these cells are exposed to adequate factors. In addition, dental pulp cells have demonstrated their multipotential differentiation ability. For instance, dental pulp cells have the capacity to differentiate into adipocytes and are characterized by mesenchymal stem cell markers (Stro-1 and CD146), and endothelial and smooth muscle markers [115, 116]. In mice, mesenchymal cells from incisors can differentiate into odontoblasts, osteoblasts and chondrocytes [117].

Other experiments indicate that there is a continuous source of progenitor cells in the dental pulp located around the blood vessels of the pulp [118]. In this context, pericytes co-express Stro-1 and pericyte-associated antigen 3G5 [119], which suggests that pericytes can be considered as stem cells of dental pulp or that pericytes are the same entity. In addition, bone production has been obtained in a subpopulation of dental pulp cells [120,121]. Very recently, adult human dental pulp cells have shown the capacity of differentiating into active neurons [122].

Dental Epithelial Stem Cells

Dental epithelial cells exert important functions during growth and development of teeth, especially in ameloblasts differentiation and enamel formation. However, all dental epithelial derivatives, including ameloblasts, are lost after tooth eruption. In consequence, it is not possible to regenerate enamel layer naturally after caries or teeth injury. Some mammals have incisors or molars that grow continuously. These teeth provide a source of interesting models for the study of mechanisms for dental epithelium regeneration. It is thought that the epithelial stem cells of rodent incisors reside in the stellate reticulum core of the cervical loop, which is located at the junction of the inner dental epithelium and outer dental epithelium, Fig. (**4**), stem cell proliferation of the cervical loop is stimulated by FGF3 of the adjacent mesenchyme, but mesenchymal BMP4 represses FGF3 expression. However, Activin inhibits the repressive effect of BMP4 on the labial side and FGF3 increases stem cell proliferation and the population of labial stem cell niches. In addition, BMP4 has opposite roles on the proliferation and differentiation of dental stem cells: it inhibits the proliferation of stem cells, whereas it stimulates their differentiation into ameloblasts on the labial side.

On the lingual side, Follistatin inhibits BMP4 and the inner dental epithelium does not differentiate into ameloblast [123-125]. It has been recently demonstrated that sprouty genes control FGF signaling [107].

Dental Follicle Stem Cells

The dental follicles are formed by mesenchymal cells that have been considered as a multipotent tissue because of its ability to generate cementum, bone and peridontal ligament.

Peridontal ligament has a certain degree of regenerative ability after injury. These cells can de differentiated into cementoblasts, adipocytes or fibroblasts under defined culture conditions.

Tooth Regeneration

It has been shown that dental components can be also formed from stem cells of non-dental tissues. For instance,

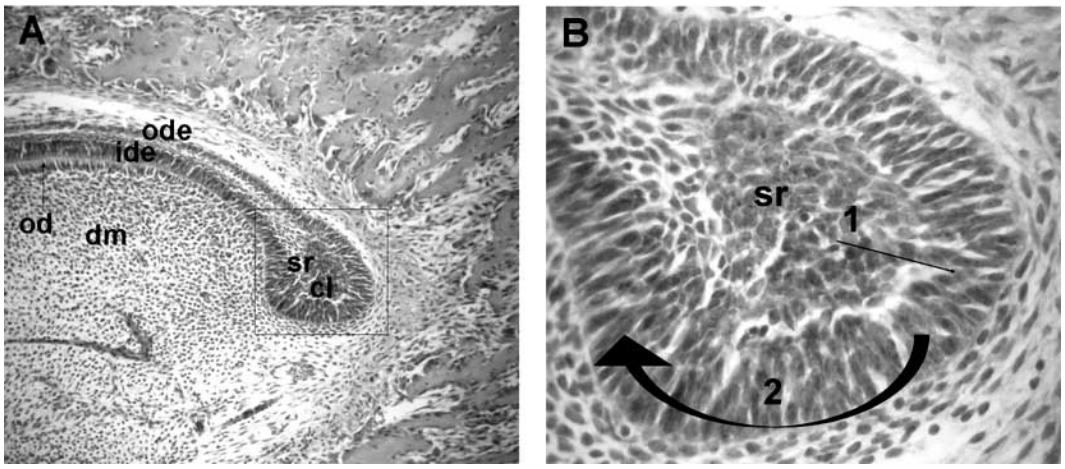

Fig. (4). The epithelial stem cell niche in mouse incisor. Pool of stem cells are found in the stellate reticulum. These cells are incorporated into the inner dental epithelium (arrow 1), the cells follow the gradient of growing of incisor (arrow 2) and will differentiate into ameloblasts depositing enamel. od: odontoblasts; cl: cervical loop; sr: stellate reticulum; ide: inner dental epithelium; ode: outer dental epithelium; cl: cervical loop; dm: dental mesenchyme.

stem cells derived from bone-marrow or neural origin have the ability to express dental genes and produce tooth structures [126, 127]. In this context, enriched bone-marrow cells can be differentiated into ameloblasts [128].

Finally, aggregates of dissociated cells of different origin, recombination of tooth tissues, organ tissue cultures and transplants into recipient animals, and the use of stem cells from embryo and adult tissues make it possible to recreate *In vivo* conditions to build specific tooth structures, (both soft and hard tissues) to regenerate, repair or replace dental tissues for future tooth therapy.

5. APPLICATIONS AND LIMITATIONS OF STEM CELLS IN REGENERATIVE MEDICINE AND FUTURE PROSPECTS

Due to presents limitations presented by stem cells in adult tissues, the most promising in the field of regenerative medicine is found in Embryonic Stem Cells (ESC) obtained from the inner cell mass of the embryo during the blastocyst stage (5 days post-fecundation), the cells have demonstrated longevity in culture by maintaining their undifferentiated state for at least 80 passages, Fig. (**5**). In addition ESC can be relatively easily grown using published protocols [129]. Moreover if ESC has a culture with the appropriate nutrients at its disposal ESC can potentially give rise to all cell types of the body including germinal cells (pluripotential) and their offspring can in turn, become integrated in a tissue, adopting the character and behaviour of the cells in this new tissue environment. The only vertebrate from which pluripotential ESC have been obtained is the mouse, and only certain strains [17]. Experiments with rats indicate that it is possible in the future to use these ESC cells already specialised to replace cells in tissues. There is now a growing array of mouse and human cell lines with pluripotent capacity, but their relationship to each other and to their lineage progenitors in the embryo is less than clear. Protocols developed in mouse ES cells may not be easily transferable to human ES cells if they are not equivalent in developmental status. Response to differentiation protocols will depend on the initial

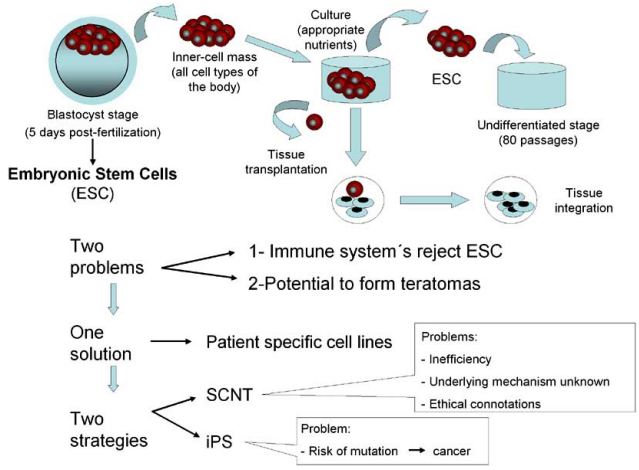

Fig. (5). Problems and limitations of ESC in the field of regenerative medicine. ESC obtained during blastocyst stage, cultured by appropriate nutrients can give rise to all cell types of body. Stem cell transplants can be used to restore tissue. SCNT: somatic cell nuclear transfer. iPS: induced pluripotential stem cells.

developmental state of different pluripotent cell lines [130]. Nevertheless, in spite of the fact that, mouse and human ESCs may represent different stages of development and obviously need different conditions for growth and maintenance in the undifferentiated state [131]. On the other hand, the signalling pathways that regulate human ESC differentiation are those that regulate these processes in mouse ESC cultures [132]. Cells with similar properties to those of rat ESC can be obtained from early human embryos creating an unlimited potential of cells that can be used to replace and repair damaged tissues. Nevertheless their use and manipulation involves legal and ethical issues [133]. Moreover there are two problems associated with the use of ESC to replace tissues:

1. If the transplanted cells differ genetically from the cells of the patient, the immune system of the latter may reject and destroy these cells.
2. Pluripotent stem cells present a safety concern because of their potential to form tumours. When these cells are transplanted in the undifferentiated state they form teratomas, tumours derived from all three germ layers. Currently, the only way to ensure that teratomas do not form is to differentiate the ESCs, enrich for the desired cell type, and screen for the presence of undifferentiated cells [134].

These problems could be avoided using two strategies to obtain "patient specific" cell lines:

1. The denominated Somatic Cell Nuclear Transfer (SCNT) also called "Therapeutic cloning" or Nuclear Cloning (NC).
2. Induced pluripotent stem cells by reprogramming adult somatic cells (iPS).

These "patient specific" cell lines have the potential of revolutionising medicine since one of the ambitions of regenerative medicine is to produce genetically equivalent (isogenic) cells. The resulting ESC are perfectly matched to the patients immune system and no immuno-suppressants would therefore be required to prevent rejection.

Somatic cell nuclear transfer (SCNT) entails the removal of an oocyte nucleus followed by its replacement with a nucleus derived from a somatic cell obtained from that patient. Activation with chemicals or electric shock stimulates cell division up to the blastocyst stage at which time the inner cell mass is isolated and cultured, resulting pluripotent stem cells to form cells genetically identical to the patient [135].

While promising, somatic cell nuclear transfer technology has certain limitations requiring further improvement before it can be applied widely in clinical practice. In addition to the ethical issues surrounding the use of human oocytes and embryos created for research [136], the scarcity of donated mature human metaphase II oocytes of high-quality (fresh, from young donors) available for research is a significant impediment [137,138]. Currently, the efficiency of the overall cloning process is quite low, as the majority of embryos derived from animal cloning do not survive after implantation [139]. To improve cloning efficiencies, further improvements are required in the multiple complex steps of nuclear transfer such as enucleation and reconstruction, oocyte activation, and synchronisation of cell cycle between

donor cells and recipient oocytes [140]. The transfer of a somatic nucleus to an enucleated oocyte provides it with a source of genetic information; the oocyte cytoplasm must effect several essential changes. The somatic nucleus must cease expression of its unique repertoire of gene products; this requires that the somatic nucleus responds to instructions from the oocyte cytoplasm in order not only to down regulate its existing expression profile but also to up regulate many long repressed genes in the correct spatio-temporal manner. Also, the heritable memory endowed by chromatin architecture of the somatic nucleus that ensured the mitotic production of identical daughter cells from that somatic cell type must be erased. This requires extensive remodelling not the underlying DNA sequence information but of the epigenetic features that control the use of this information by the cell [137]. Other factors to keep in mind that can help to improve the cloning efficiency, oocyte-derived mtDNA (mitochondrial DNA) was considered to be a potential source of immunologic incompatibility. Differences in mtDNA-encoded proteins expressed by cloned cells could stimulate a T-cell response minor histocompatibility antigens, that could prevent the use of these cloned constructs in patients with chronic rejection of major histocompatibility matched human renal transplants. Nevertheless, analyses revealed that the cloned renal cells showed no evidence of a T-cell response, suggesting that rejection will not necessarily occur in the presence of oocyte-derived mitochondrial DNA [141]. Another open question whether the differentiation state of donor cell affect the efficiency of the cloning process, the generation of cloned cells from neurons was less efficient than that from neural stem cells [142]. However, because the cloning process is affected by many other parameters, such as cell cycle and the physical characteristics of the donor nucleus, it has remained unresolved [143]. In spite of these barriers, French *et al.* [144], reports for the first time, that SCNT can produce human blastocyst stage embryos using nuclei from a differentiated adult somatic cell. Mature oocytes, obtained by transvaginal aspiration, were enucleated using extrusion or aspiration. Somatic cell nuclear transfer (SCNT) embryos were constructed using two established adult male fibroblast lines of normal kariotype. One cloned blastocyst was confirmed by DNA and mtDNA fingerprinting analyses and DNA fingerprinting of two other cloned blastocyst indicated they were also generated by SCNT. However, the results verified, for the first time through DNA and mtDNA fingerprinting that of the 5 blastocysts only one had the donor cell genomic DNA and the oocyte mtDNA. Considering such a small number of blastocysts, the experiment could have rendered zero clones [145].

At the present time, the medical applications of clonation have been halted on account of the inefficacy of the process, the lack of knowledge of the underlying mechanism, and ethical concerns. One of the key issues raised by nuclear cloning relates to the reprogramming that convert the epigenome of a somatic cell into that of an embryonic cell. Several strategies such as nuclear transplantation, cellular fusion, and culture induced reprogramming have been employed [146]. From then Takahashi and Yamanaka [147] have successfully reprogrammed mouse embryonic fibroblasts and adult fibroblasts to pluripotent ES-like cells after viral-mediated transduction of the four transcription factors Oct4, Sox2, c-myc, and Klf4. Some of the transcription factor have been linked with oncogenesis and retroviral insertion alone can cause deleterious and cancer–causing mutations [10]. This makes questionable any possible application of iPS in regenerative medicine, especially cell therapy. The recent reports on direct reprogramming of human skin fibroblast to induce pluripotent stem cells (iPS) with an ES-like cell phenotype are of particular because if derived from patients carrying gene mutations affecting the cardiovascular system, it should be possible to obtain cardiac progenitors with the same mutations. This should enable study at the cellular level, allowing molecular and genetic screens to find drugs to halt or reverse the disease phenotype [132, 146, 148].

ES cells can be derived from patients with specific diseases and protocols can be established to direct the disease-specific ES cells to become the very types of cells affected the disease. Such disease-relevant cells can bee tools for the analysis of the disease mechanism and should be able to drive more predictive drug discovery and toxicity studies [149].

Clearly, a number of problems, both ethical and technical, need to be surmounted if it going to lead the successful application of patient-specific NTSC o iPS in regenerative medicine. However, stem cells may also transform the way in which therapeutics are discovered and validated.

ACKNOWLEDGEMENTS

We thank David Halett for review of this manuscript. This work was partially supported by grants from the University of the Basque Country (UPV06/99), Fondo de Investigación Sanitaria (FIS) of Spanish Ministry of Health (PI060908) and from the Basque Government (IT-287-07).

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Received: July 07, 2008 Revised: February 09, 2009 Accepted: May 08, 2009