Slovak J. Anim. Sci., 43, 2010 (2): 50 - 59
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ISSN 1337-9984

Minireview

EMBRYONIC STEM CELLS - CURRENT KNOWLEDGE AND FUTURE PROSPECTS

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ABSTRACT

Embryonic stem cells *bona fide* encompass cells of the inner cell mass, explanted from blastocysts, that exist exclusively in *in vitro* culture systems. To qualify for a stem cell, one has to be capable of self-renewal, multilineage differentiation and functional re-population of a target tissue. Besides essential aspects of stem cell biology, in this review, we discuss categories according to the differentiation potential or plasticity of stem cells which is closely intertwined with their developmental rank in mammals ranging from totipotent zygote to monopotent stem cells. Aspects of embryonic stem cells, their isolation, signalling pathways governing their self-renewal and pluripotency, markers, genetic modification and differentiation are reviewed. Finally, we outline some hurdles, challenges and outlooks in the field of stem cell research and its promises for regenerative and replacement therapies.

Key words: ESC; self-renewal; pluripotency; totipotency; marker; gene targeting; gene trapping; differentiation; stem cell therapy

INTRODUCTION

Basic characteristics of stem cells

Three basic categories of cells make-up the human body: germ cells, somatic cells and stem cells. Somatic cells include the bulk of the cells that make-up the human adult and each of these cells in its differentiated state has its own copy, or copies, of the genome; the only exception being cells without nuclei, i.e. red blood cells. Germ cells are cells that give rise to gametes, i.e. eggs and sperm (Alison et al., 2002).

According to Weissman (2000) there are 3 criteria that cells need to satisfy in order to qualify for a stem cell: 1. They need to be capable of self-renewal, *i.e.*undergoing symmetric or asymmetric divisions by means of which the stem cell population is maintained. Daughter cells that emerge from symmetric division retain full stem cell characteristics, whereas asymmetric division generates only one stem cell, the other sets out for a differentiation pathway. 2. A single cell must be capable of multilineage differentiation. 3. Capacity for *in vivo* re-population and functional reconstitution of a given tissue.

Stem cells are able to differentiate into cell types beyond the tissues in which they normally reside. This is often referred to as stem cell plasticity. Stem cells are also believed to be slow cycling but highly clonogenic and generally represent a small percentage of the total cellular make-up of a particular organ (Gardner, 2002).

Developmental hierarchy of stem cells according to their differentiation potential

Totipotent stem cell gives rise to both embryo and placenta. The physiological totipotent stem cell is a fertilized oocyte (zygote) or first blastomeres. The

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Received: March 22, 2010
Accepted: March 24, 2010

artificial counterpart is a clonote obtained by nuclear transfer to an enucleated oocyte. When the blastomeres have divided to form the 32-cell stage, the embryo is known as a morula. Cells that form the morula have already lost their totipotency and are now pluripotent (Ratajczak et al., 2008).

During early embryonic development, stem cells undergo specification giving rise to extraembryonic endoderm and to the cells of the inner cell mass (ICM). These cells are pluripotent but no longer totipotent as they can no longer give rise to extraembryonic cell types (Verfaille, 2009). The pluripotent stem cell (PSC) is defined as being able to contribute to the development of the embryo but having lost the capacity to form the trophoblast (which gives rise to the placenta). Pluripotent cells are only surpassed in their developmental capacity by the truly „toti-" or „omnipotent" blastomeres from the early morula. However, blastomeres have not been shown to proliferate indefinitely and are therefore not classified as stem cells. In addition to the ICM, epiblast stem cells (EPSC) and stem cells obtained as immortalized cell lines – blastocyst-derived embryonic stem cells and PGC-derived embryonic germ cells (EGC) are pluripotent sa well (Ratajczak et al., 2008).

Cells in the ICM specify sequentially to epiblast cells, primitive ectoderm, followed by creation of definitive ectoderm, definitive endoderm and mesoderm and ultimately to tissue specific stem cells. As specification progresses, cells become more and more limited in their differentiation ability, so that the stem cells present in tissues of the three germ layers can only generate cells of that tissue but no longer cells of other tissues within the same germ layer or the other germ layers. These cells are therefore termed multipotent.

Spermatogonial stem cells produce only one type of mature cells – spermatogonia (Verfaille, 2009). Such cells are generally referred to as monopotent stem cells. Monopotent stem cells are tissue commited stem cells that give rise to cells of one lineage, *e.g.* hematopoietic stem cells, epidermal stem cells, intestinal epithelium stem cells, neural stem cells, liver stem cells or skeletal muscle stem cells (Ratajczak et al., 2008).

A scheme of the stem cell developmental hierarchy is depicted in the Figure 1.

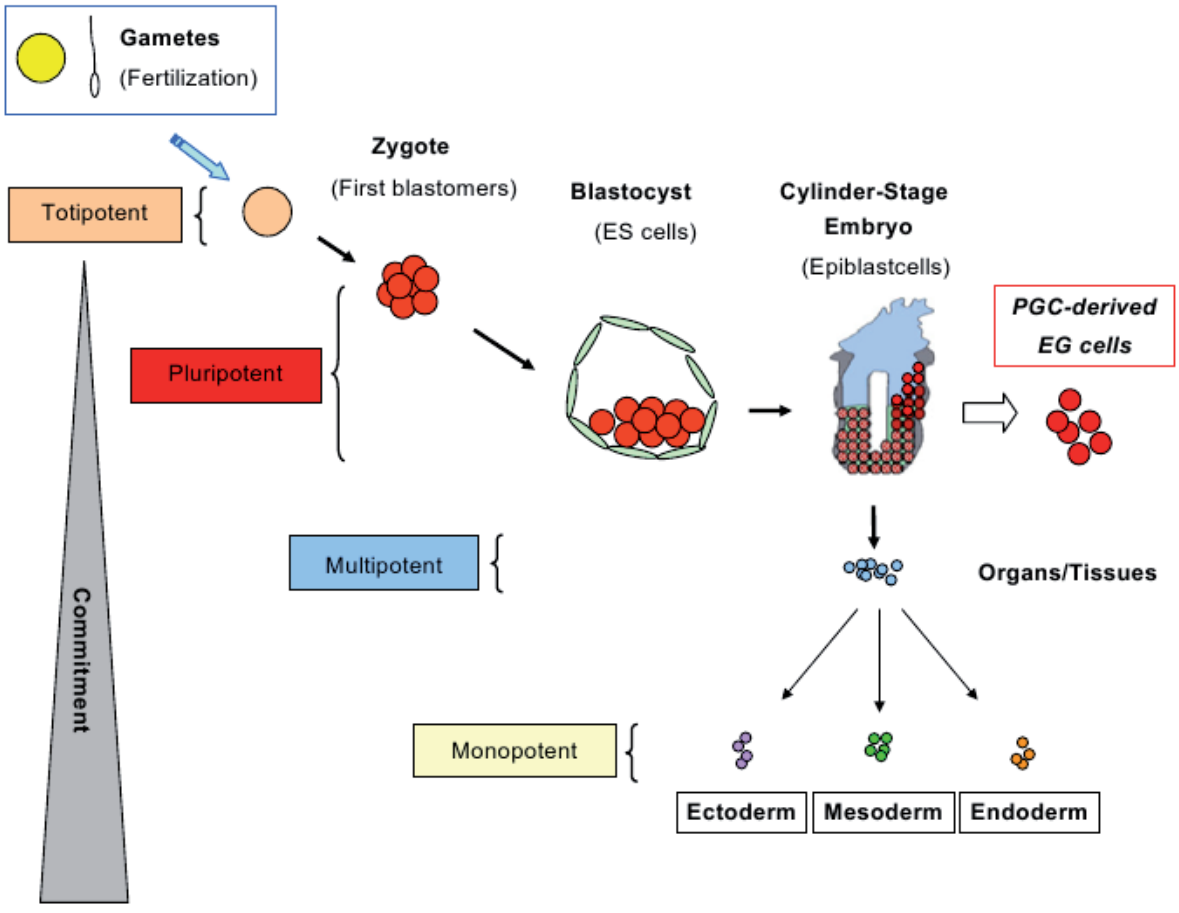

Fig. 1: Developmental hierarchy of the stem cells according to their differentiation potential (RATAJCZAK et al., 2008)

Embryonic stem cells

In contrast to multipotent stem cells found in tissues and organs, cells in the ICM of the blastocyst are pluripotent, as was shown by Gardner and Edwards (1968), when he created chimeric animals by transferring cells of the ICM from one blastocyst into another. In 1981, two groups demonstrated that when ICM cells are plated onto mouse embryonic fibroblasts (MEF), continuously growing cell lines termed embryonic stem cells (ESC) can be established while retaining features typical for pluripotent cells (EVANS and Kaufman, 1981; Martin, 1981). Pluripotency of ESC can be demonstrated *in vitro* by allowing the cells to self-assemble in cell clusters or embryoid bodies (EB), without support of MEF. ESC then undergo spontaneous differentiation to many cell types of the three germ layers. Pluripotent stem cells will generate teratomas *in vivo*, wherein small organoid structures are found representing the three germ layers, and in case of mouse ESC, final proof of pluripotency is the generation of chimeric mice, wherein the donor ESCs contribute their share to somatic as well as germ cell populations.

It was not until the late 1990s that ESC were also generated from higher mammalian species, first from blastocysts of non-human primates (Thomson et al., 1995) and subsequently from human blastocysts (Thomson et al., 1998). Stable embryonic stem cell lines were also derived from rabbit blastocysts (Honda et al., 2008). Although many previous attempts have been made to derive ES cell lines from rabbits, none has been successful (Cole et al., 1964; Cole et al., 1966; Graves and Moreadith, 1993; Fang et al., 2006; Wang et al., 2007). In pigs, more than 30 attempts to establish ES cells have been published, but no cell lines are available because the culture conditions required to prevent spontaneous differentiation and senescence in inner cell mass (ICM)-derived cells have not been identified (reviewed in Vackova et al., 2007). Saito et al. (2002) was successful in establishing stable embryonic stem cell lines from the equine blastocysts and was able to maintain its proliferation potential for more than 56 passages. This is a plausibility for the horse-racing industry considering the fact that the current stem cell therapy aimed at treating injuries in horses involves mesenchymal stem cells which exhibit several drawbacks (Saito et al., 2002). There are several reasons why the dog is a suitable model for the study of human diseases (reviewed in Starkey et al., 2005; Schneider et al., 2008).

Embryonic stem cell signalling pathways and markers

The factor capable of promoting self-renewal and inhibiting differentiation provided by the feeder cells was identified in the late 1980s. Originally termed differentiation inhibiting activity (DIA) (Smith and Hooper, 1987), this factor was later identified as leukemia inhibitory factor (LIF), a member of the IL-6 cytokine family (Smith et al., 1988; Williams et al., 1988). This discovery enabled propagation of ES cells free from feeder cells on gelatinised tissue culture plastic in the presence of serum and recombinant LIF (Friel et al., 2005).

The LIF receptor consists of the LIF-specific receptor subunit LIFRβ and the common signal transducer gp130 (Davis et al., 1993). The effect of LIF on self-renewal is not exclusive but is also seen by a small group of related cytokines which act via the common gp130 receptor (Yoshida et al., 1994). The engagement of LIF by its receptor mediates activation of two major intracellular signalling pathways, the JAK-Stat pathway and the SHP2-Erk pathway. Following binding of LIF to its receptor this complex activates related Janus-associated (JAK) tyrosine kinases that phosphorylate the receptor chains. A latent transcription factor STAT3, which contains a key Src homology 2 (SH2) domain, is phosphorylated by the JAKs which, in turn, promotes dimerisation of STAT3. The STAT3 dimers then translocate to the nucleus, where they bind to sites on the DNA controlling the transcription of genes important in ES self-renewal (Niwa et al., 1998).

However, there is contradictory evidence that undermines the view, that STAT3 signalling is the key event in determining the self-renewing phenotype (Berger et al., 1997). This suggests that there is an underlying feature of ES cells, that determines the ES cell phenotype, that is independent of the STAT3 pathway. Chambers et al. (2003) and Mitsui et al. (2003) described a divergent homeobox transcription factor which promotes ES self-renewal, pluripotency and epiblast formation. This gene was named Nanog, after the mythical Celtic land of the ever-young *Tír na nÓg*. Nanog is specially expressed in ES cells, EC cells and embryonic germ cells, but is not expressed in hematopoietic stem cells, adult tissues or differentiated cells Chambers et al., 2003; Mitsui et al., 2003).

Mouse ES cells have a distinct easily recognisable morphology to the trained eye including a high nuclear-to-cytoplasmic ratio and prominent nucleoli. They also express markers characterised as distinctive of pluripotent cells. These include an isozyme of alkaline phosphatase and a cell surface marker stage-specific embryonic antigen-1 (SSEA-1). While some of these proteins are not ES cell-specific, they are useful in that they can be used to distinguish between pluripotent and differentiated cells (Friel et al., 2005).

A classical marker of undifferentiated ES cells is expression of the POU class transcription factor Oct4 gene (also known as Oct3 or Oct3/4). Its expression is restricted to totipotent and pluripotent cells of the mouse (Pesce et al., 1998) and is down-regulated in the majority of adult

tissues excluding the germ line (Yeom et al., 1996). Apart from being a marker, Oct 4 is a key factor that regulates self-renewal of ES cells. Nichols et al. (1998) showed by targeted gene deletion that the role of Oct4 *in vivo* is to establish the pluripotent nature of cells within the ICM. In ES cells a critical level of expression of Oct4 is essential in maintaining ES cell renewal (Niwa et al., 2000). Less than a two-fold increase causes ES cells to differentiate into mesoderm or endoderm, while reduction to less than 50% expression levels causes differentiation into trophectoderm. Oct4 is not a direct target gene of STAT3 and therefore its expression is not directly regulated by LIF. When Oct4 is constitutively expressed within ES cells it does not prevent differentiation of ES cells induced by LIF withdrawal (Niwa et al., 2000). Thus, expression of Oct4 alone is not sufficient to maintain pluripotency, but rather also needs the cytokine-induced action of STAT3 (Friel et al., 2005).

Oct4 is thought to function in several ways within ES cells. Many ES cell-expressed genes (*e.g.* the zinc-finger protein Zfp42/Rex-1) have Oct4 binding domains within their regulatory elements and, therefore, Oct4 can directly activate these target genes. Oct4 can also activate or repress other target genes by cooperating with various transcriptional co-factors. Two such co-factors thought to play a key role in self-renewal are the Sry-related factor Sox2 (Avilion et al., 2003) and the Forkhead Box family member, FoxD3 (Hanna et al., 2002). Both factors cooperatively function with Oct4 (Guo et al., 2002; Yuan et al., 1995).

The expression pattern of numerous protein markers and signalling molecules in rabbit, human and mouse embryonic stem cells is listed below in the Table 1 adapted by Gócza and Bősze (2009).

Table 1: Molecular characterization of ES-like cell colonies

	rabESC	hESC	mESC
AP	+	+	+
SSEA-1	+	-	+
SSEA-3	+	+	-
SSEA-4	+	+	-
TRA-1-60	+	+	-
TRA-1-81	+	+	-
OCT 4	+	+	+
SOX 2	+	+	+
UTF-1	+	+	+
Nanog	+	+	+
LIFR	+	+,-	+
gp130	+	+	+
LIF	+	+	+
CD9	+	+	+

	rabESC	hESC	mESC
Nodal	-	+	+
TGF-β1	+	+	+
BMP4	+	+	+
WNT1	+	-	-
WNT2	+	-	-
WNT3A	-	-	-
WNT4	+	+	+
WNT5A	+	-	-
DKK1	+	+	+
DKK2	-	+	+
Gsk3-β	-	+	+
FGF1,2	+	+	+
FGFR 1,2,3,4	+	+	+

Genes related to pluripotency, including many genes of FGF, TGF-β/BMP, and WNT signaling, were detected in ESCs of rabbit (rabESC), human (hESC (BG02)), and mouse (mESC(R1)). Some gene expression of WNT and TGF-β/BMP signaling was different in these three species. Inhibitors Dkk2 and Gsk3-β were not expressed in rabbit ESCs (RF and RP cell lines) but were expressed in human and mouse ESCs and ligands WNT1, WNT2, WNT4, and WNT5A were expressed in rabbit ESC lines, but only WNT4 was detected in human and mouse ESCs. Nodal, a TGF signaling ligand gene, was not detected in rabbit ESCs but did express in human and mouse ESCs. Abbreviations: +, detected; −, not detected; BMP, bone morphogenetic protein; ESC, embryonic stem cell; FGF, fibroblast growth factor; TGF-β transforming growth factor-β (adapted from Wang et al. (2007) and Catunda et al. (2008) modified by Gócza and Bősze (2009)).

Genetic modification of stem cells

The past century has witnessed a boom in molecular genetics and one of the greatest projects - the human genome project (HGP) (Abramowicz, 2003). Upon accomplishment of this project the human genome sequence information has been hoarded, which can now be employed in the diagnosis of certain diseases on the level of genes (Burton and Stewart, 2003; Collins and Mansoura, 2001; Gottesman and Collins, 1994). Biological research has entered the so-called post-genomic era focusing on deciphering the function of each gene in our genome (Austin et al., 2004; Eisenberg et al., 2000). Comparative genomics has shown that the mouse and human genomes exhibit high homology (Gregory et al., 2002). Therefore, the mouse serves as a perfect model animal for human functional genomic research. The most efficient way to study the function of a gene is to make a knockout and observe the outcome for the animal‘s phenotype (Austin et al., 2004; Brown and Hancock, 2006; Dinnyes and Szmolenszky, 2005). Knock-out mice can also be used as model organisms for the research on human diseases.

There are two main methods to make knock-out mice: gene targeting and gene trapping. Gene targeting technology is based on ES cell culture and *in vitro* homologous recombination, and it is a method of gene knock-out and knock-in in the mouse (Hogan and Lyons, 1988). The first mouse gene targeting experiment was conducted in 1987 (Mansour et al., 1988; Thomas and Capecchi, 1987). Gene targeting has accelerated the research on gene function in the last 20 years elucidating crucial mechanisms involved. In 2007, three scientists were awarded of the Nobel Price for their research contribution into the technology of gene targeting (Mak, 2007).

Gene trapping mutagenesis was developed as an alternative to gene targeting technology. It is a high-throughput and random mutation technique (Abuin et al., 2007; Gossler et al., 1989; Kothary et al., 1988). Though, not as specific as the gene targeting, a large number of mouse genes can be knocked out in a short period of time by trapping (Takeuchi, 1997; Zambrowicz and Friedrich, 1998).

The combination of gene trapping and gene targeting makes it possible to knock out all the mouse genes. Improvements have also been made to overcome shortcomings in the two techniques (Guan et al., 2010).

Laboratory rabbits have long been used in a biomedical research. Transgenic rabbit models, created through routine microinjection method, are widely used as experimental models of human diseases such as atherosclerosis, myocardial infarction, hypercholesterolemia, hypertension, bone and cartilage disorders (BŐSZE and Houdebine, 2006; Shiomi and Fan, 2008). Rabbit ES cells would be invaluable both for creating second generation transgenic models of human diseases using gene-targeting technology and for testing stem cell therapies for human applications (Gócza and Bősze, 2009).

Genetically modified rabbit ESCs have recently been generated by two authors - Fang et al. (2006) and Honda et al. (2008), as a first step towards producing transgenic rabbits from these cells. Fang et al. (2006) used the technique of electroporation to embed the gene construct based on a pCX-mRFP-neo plasmid into the target rabbit stem cells dispersed in a suspension in a culture medium. Honda et al. (2008) used the technique of transduction of self-inactivating lentiviral vector construct pCS-CDF-UbC-GFP-PRE, which contains the green fluorescent protein gene under the control of human ubiquitin C.

Stem cell diferentiation

The potential of embryonic stem cells to differentiate into almost all cell types, in addition to providing unlimited number of cells, has stirred interest in their use as an integral part of modern clinical treatment (Hwang et al., 2008). Additionally, stem cells are being used to understand the complex molecular and cellular events occurring during early development, disease progression, epigenetics, and pathophysiology (Nishikawa et al., 2007; Jakobsson et al., 2007; Giorgio et al., 2007).

Perhaps the most exciting of all applications of stem cells could be their use in cell replacement therapies and regenerative medicine. The chronic shortage of organ transplants in conjunction with the limitation of artificial implants (prostheses) has intensified research in cell and tissue based therapies. The key advantage of cell and tissue therapy over pharmacological therapies to treating debilitating diseases and abnormalities is that the former offers “living biological replacements” while the latter merely provides a palliative solution. However, before stem cell-based therapies could be transferred into practice, many fundamental biological and engineering challenges need to be overcome, that include: controlling the self-renewal of stem cells, directing the lineage/tissue-specific stem cell differentiation, *in vivo* delivery, and integration to the host milieu (Hwang et al., 2008).

Differentiation of the ES cells prior to transplantation is very critical, because undifferentiated ES cells may cause teratoma formation *in vivo*. The potential use of ES cells to replace functional loss of particular tissues may depend on efficient differentiation protocols to derive tissue-specific progenitor cells without any detrimental *in vivo* side effects. By manipulating the culture conditions in which ES cells differentiate, it has been possible to control and restrict the differentiation pathways and

thereby generate cultures enriched in lineage-specific precursors *in vitro*. However, commitment and long-term engraftment of these cells *in vivo* for functional tissue regeneration are challenging (Hwang et al., 2008). In addition, the intrinsic biological difference between somatic cells and hESC-derived somatic cells may exist. In a recent report, Mauck et al. (2006) suggested stem cell-based engineered tissues are likely to be an inferior tissue. It is necessary to design and develop culture conditions that promote homogeneous and enhanced differentiation of ES cells to yield functional tissues (Hwang et al., 2008).

Different strategies have been utilized to induce *in vitro* differentiation of ES cells. ES cells spontaneously differentiate into derivatives of three embryonic germ layers: mesoderm, endoderm, and ectoderm via formation of embryoid bodies (EBs) upon removal of factors that maintain the undifferentiated or pluripotent state of stem cells (Weitzer, 2006).

Creation of EBs is usually the first step for differentiation of ES cells. Differentiation of EBs into particular cell lineages has been extensively studied due to current technical challenges in achieving their homogenous and efficient differentiation (Kurosawa, 2007).

Stem cell differentiation is context dependent. Even though EBs have a three-dimensional structure, terminal differentiation of EBs is conducted in 2D culture (tissue culture plates). Most of the studies investigating stem cell differentiation have been performed on 2D plates coated with various biomaterials. Precise spatial and temporal presentation of factors directing the stem cell differentiation is extremely important to achieve homogeneous and efficient differentiation. ES differentiation in 2D cultures does not mimic the physiological (*in vivo*) environment and may result in inefficient and heterogeneous differentiation. Indeed, significant differences were found in the differentiation profile of ESCs when cultured in a 3D environment vs. 2D (Tanaka et al., 2004; Hwang et al., 2006). Three-dimensional cultures in the form of pellets alone are sufficient to induce selective differentiation of embryonic-derived cells (Kim et al., 2005; Liu and Roy, 2005). Maintenance and differentiation of EBs in three-dimensional culture may promote cell–cell interactions, entrapment of secreted extracellular matrix, and maintenance of spherical cellular morphologies (Liu and Roy, 2005; Liu et al., 2006). In addition, 3D culture and differentiation of ES cells provide structural support for higher order tissue organization and remodeling (Hwang et al., 2008).

Advancements in stem cell biology have enabled the use of EBs to produce unlimited numbers of specialized progenitor cell populations for stem cell-based therapy. One hypothesis for such applications is, that partially differentiated or tissue-restricted progenitor cells can be isolated from the ES cells, purified through cell selection, and expanded *in vitro* to generate adequate progenitor cell populations before they can be used safely and effectively in clinical applications. ES cells differentiate into multiple mature somatic cell types, presumably via precursor cells, when the appropriate stimuli are applied (Hwang et al., 2008). For instance, mesodermal progenitor cells were isolated by fluorescence-activated cell sorting (FACS) after EB stimulation with BMP (Nakayama et al., 2003). Moreover, multipotent hematopoietic progenitor cells (Wang et al., 2005; Ma et al., 2007), cardiac progenitor cells (Moretti et al., 2006; Baba et al., 2007), endothelial progenitor cells (Levenberg et al., 2002), and neuronal progenitor cells (Brokhman et al., 2008) have been isolated and characterized. Recently, LU et al. (2007) have reported an efficient and reproducible method for generating large numbers of such bipotential progenitors (hemangioblasts) from hESCs using an *in vitro* differentiation system.

Future prospects of stem cell research

Stem cell research has now become one of the most rapidly growing areas of biomedical research internationally. A number of experimental challenges that seemed insurmountable only a few years back, reprogramming of somatic cells with defined factors being but one example, have been met. The experience from decades of molecular genetic studies of embryonic development has proven remarkably useful in efforts to guide human embryonic stem cells toward desired differentiation fates. Application of genome-wide approaches to stem cell transcription and epigenetics has provided a blueprint of the networks that control pluripotency. And gradually, discoveries from basic research are moving into the translational arena.

Many pre-clinical translational studies in a variety of disease or injury models have shown that the administration of stem or progenitor cells results in benefit, but in most studies, it is uncertain whether the improvement seen is a result of functional integration of the graft into the damaged host tissue or an indirect effect of survival or trophic factors secreted from the grafted cells. In a spinal cord injury model in the mouse, Anderson (University of California Irvine) showed that human neural stem cells could form myelinating oligodendrocytes and neurons that made synaptic connections with host cells. Inoculation *in utero* of mesenchymal stem cells into oim mice, a model of osteogenesis imperfecta, increased bone strength and reduced spontaneous fracture rates, and descendants of the grafted cells were found at areas of bone remodeling and fracture repair (Fisk, Imperial College London). Several groups reported early improvement of function

in animal models of myocardial infarction or pacemaker dysfunction after administration of ES-cell derived cardiomyocytes (Gold, Geron Corp.; Gepstein, Technion-Israel Institute of Technology), but achievement of long-term functional improvement attributable to integration of the cells into host myocardium still presents challenges (Mummery, Hubrecht Institute) (Orkin and Pera, 2007).

Much of the current research into stem cell biology is focused on its potential for regeneration of various tissues and organs. Stem cell-based therapy with autologous bone marrow stem cells could provide an attractive alternative to the classical therapeutic approach in the foreseeable future. The possibility of nervous tissue regeneration in neurodegenerative disorders of the central nervous system generates a special challenge for researchers and clinicians involved in that field of medicine (Paczowska et al., 2009). Very small embryonic-like stem cells (VSEL SCs) (Lu and Miao, 2008; Ratajczak et al., 2008), recently discovered in murine bone marrow and human umbilical cord blood, arouse great hope. VSEL SCs display several features typical for embryonic stem cells, such as a large nucleus surrounded by a narrow rim of cytoplasm, euchromatin, and expression of pluripotent markers (Oct-4, Nanog, SSEA-4). Application of these cells in regenerative medicine could have considerable advantages over strategies using embryonic stem cells, since ethical concerns might be naturally solved. Thus, these cells can become a recommended source of stem cells for cell therapy as compared to those isolated from developing embryos (Paczowska et al., 2009).

One of the most common diseases of the pancreas is *diabetes mellitus*. The current treatment of exogenous insulin supply is not fully capable of achieving tight control of glucose regulation, leading to long-term complications. Hence, recent success in islet transplantation-based therapies for *diabetes mellitus* and the extreme shortage of pancreatic islets have motivated recent efforts to develop renewable sources of islet-replacement tissue developed using the strategies of stem-cell based regeneration (Hori, 2009).

The latest research reports revealed the presence of stem/progenitor cells located in different regions of matured eye. They are able to differentiate into retinal pigment epithelium cells as well as neural structure of retina. These cells were identified in neurosensory retina, pigment epithelium and within cilliary body and iris epithelium. Moreover, it has been proved that Muller glia possess the potential of differentiation into retinal cells. These findings indicate the presence of potential mechanisms enabling retinal cell re-population and retinal tissue regeneration (Machalińska and Zuba-Surma, 2009).

The generation of ES cell lines bearing mutations that confer a predisposition to disease could give insight into the process by which the disease arises and potentially lead to the development of new therapies. These cell lines could either be generated by making targeted mutations or by using nuclear transfer from cells isolated from affected individuals. These ES cells could then be differentiated into affected cell types to study the development and progression of the disease and could also be used to screen new drugs or to evaluate new therapies *in vitro* or *in vivo*, for instance after transplantation into immunocompromised mice. For example, while Alzheimer's disease may affect several cell types, unlike PD or type I diabetes, the generation of cholinergic neurons containing mutations predisposing them to Alzheimer's disease could aid in a better understanding of the development of the disease, as well as facilitate the discovery of new therapies (Mayhall et al., 2004).

ACKNOWLEDGEMENT

This work was supported from the grants of Slovak Research and Development Agency: APVV LPP-0119-09.

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