Chapter from the book *Methodological Advances in the Culture, Manipulation and Utilization of Embryonic Stem Cells for Basic and Practical Applications*

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1. Introduction

A stem cell is a specific kind of cell that has the unique capacity to renew itself and to give rise to specialized cell type.

In terms of potentially, stem cells can be classified in three types:

- Totipotent: is the ability to form all cell types, including the extra-embryonic tissues. In mammals, the fertilized egg, zygote and the first 2, 4, 8, 16 blastomeres from the early, are examples of totipotent cells.
- Pluripotency: is the ability to differentiate into several cell types derived from any of the three germ layers (ectoderm, mesoderm, endoderm), but they are unable to produce extra-embryonic tissues. Cells from the inner cell mass of blastocyst are pluripotent.
- Multipotent: cells can form a small number of tissues that are restricted to a particular germ layer origin: e.g. blood cells or bone cells.

In according to their source, stem cells are categorized in embryonic or adult (Fig. 1):

- Embryonic stem cells (ES cells) are derived from the inner cell mass of the blastocyst (an early stage embryo) and have a high proliferative capabilities and differ from other stem cells because they have the ability to generate derivatives of all three germ layers. Embryonic stem cells have been shown to contribute to all cell lineages, including the germ line, following microinjection studies in murine embryos which give rise to chimeras (Bradley et al., 1984; Nagy et al., 1990). In vitro, murine ES cells can be propagated indefinitely in an undifferentiated state, under specific culture conditions they can differentiated into specific cell types.
- Adult stem cells are undifferentiated cells found among differentiated cells of a specific tissue, including bone marrow (de Haan, 2002), skin (Watt, 2001), intestinal epithelium (Potten, 1998), liver (Theise et al., 1999), retina (Tropepe et al., 2000), central nervous system (Okano, 2002), pancreas (Ramiya et al., 2000) and skeletal muscle (Seale et al., 2001). They typically can differentiate into a relatively limited number of cell types.

There is no doubt that stem cells have the potential to treat many human afflictions, including cancer, diabetes, neurodegeneration, as well as for studying basic developmental biology, and intensive screening of drug and toxic (Watt and Driskell, 2010).
2. Derivation of mouse embryonic stem cells

Embryonic stem cells are derived from the inner cell mass (ICM) of the mammalian blastocyst. The first mammalian ES cell lines were derived from mouse blastocyst in 1981 from two independent groups (Evans and Kaufman, 1981; Martin, 1981). One distinct property of ES cells is that they remain diploid even after being cultured for many weeks. This is in contrast to other tissue culture cell lines that often do not remain diploid but spontaneously gain or lose chromosomes at high rate. A second unique property of ES cells is that they remain pluripotent and maintain the ability, like ICM cells, to form chimeras. These two properties, maintaining normal karyotype and extensive contribution in chimeras, are both necessary for ES cells to form functional germ cells in chimeras (Sedivy and Joyner, 1992) and, moreover, have made ES cells a unique tool for gene targeting and generation of genetically modified mice.

A surprising feature of mouse ES cell lines is that the majority of cell lines genetically tested are of male origin (40XY). In female (XX) ES cells, both X chromosome are active, that may result in the unsuitable propagation of ES cells (Rastan and Robertson, 1985). In either case, the XY genotype confers appreciable advantages for germ line transmission.

ES cells clonally derived from a single cell could differentiate into a variety of cell types in vitro and form teratocarcinomas when injected into mice (Martin, 1981). Most important, cells karyotypically normal contribute at a high frequency to a variety of tissue in chimeras, including germ cells, thus providing a practical way to introduce modifications to the mouse germline (Bradley et al., 1984).
After the first derivation of mouse ES cell lines from blastocysts, several standard protocols were developed (Robertson, 1987; Abbondanzo et al., 1993; Hogan et al., 1994; Nagy et al., 2003). The efficiency of mouse ES cell derivation is strongly influenced by genetic background. For example, ES cells can be easily derived from the inbred 129/ter-Sv strain but less efficiently from the C57BL/6 strain (Ledermann and Burki, 1991). However, mouse ES cells can be derived from some non-permissive strains using modified protocols (McWhir et al., 1996; Bryja et al., 2006a; Bryja et al., 2006b). Mouse ES cells have also been derived from cleavage stage embryos and even from individual blastomeres of two- to eight-cell stage embryos (Chung et al., 2006; Wakayama et al., 2007).

ES cells or ES cell-likes have been produced in other animal models, including: medaka fish from midblastula stage (Hong et al., 1998), zebrafish from midblastulae stage (Sun et al., 1995), chickens from stage X blastoderm (Pain et al., 1996), hamsters (Doetschman et al., 1988), mink (Sukoyan et al., 1992), rabbit (Schoonjans et al., 1996), cattle (Cibelli et al., 1998; Strelchenko et al., 2004), sheep (Wells et al., 1997), and pigs (Li et al., 2003), however, only mouse and chicken ES cells are capable of colonizing the germ line.

3. Maintenance of mouse embryonic stem cells

ES cells can be stably propagated indefinitely and maintain a normal karyotype without undergoing cell senescence in vitro when cultured in the presence of leukemia inhibitory factor (LIF) and, depending on ES cell lines, with or without a layer of mitotically inactivated mouse embryonic fibroblasts (MEFs). LIF, a member of the IL-6 family, is known to strongly promote self-renewal in ES cells (Smith et al., 1992). LIF binds to LIF receptor (LIFR) to dimerize with interleukin 6 signal transducer (gp130), resulting in the phosphorylation of signal transducer and activator of transcription 3 (Stat3) via Janus kinase (Jak) activation (Burdon et al., 2002). Phosphorylated Stat3 dimerizes and translocates to the nucleus to activate a variety of downstream genes. Repression of Stat3 results in differentiation (Niwa et al., 2009), whereas artificial activation of Stat3 is sufficient to maintain pluripotency without LIF in the media (Matsuda et al., 1999).

![Fig. 2. Fluorescent immunostaining of undifferentiated mouse ES cells. All the undifferentiate ES cells expressed pluripotency specific marker Oct4. Immunostaining with DAPI (nuclear marker), Oct4 antibody, merge DAPI/Oct4](https://www.intechopen.com)

In combination with the LIF-Stat3 pathway, the pluripotency of ES cells is modulated by transforming growth factor β (TGFβ) superfamily members. These include Bmp and Activin, which generally play diverse roles in cellular homeostasis. In the ES cells, Bmp4 activates the MAD homolog 1 (Smad1). This upregulates the expression inhibitor of DNA-binding genes (Id), which suppress differentiation in combination with the LIF signal.
Activin/nodal signaling contributes to promote the growth of ES cells (Ying et al., 2003; Ogawa et al., 2007; Wu and Hill, 2009). Wnt signalling also contributes to the maintenance of pluripotency. In the canonical Wnt pathway, the Wnt receptor Frizzled transduces the signal to glycogen synthase kinase 3β (GSK3β) and adenomatosis polyposis coli (Apc). This enables catenin beta 1 (Ctnnb1) to traslocate into the nucleus to form the Ctnnb1/Tcf complex, which in turn activates the downstream genes (Willert and Jones, 2006). In the presence of Wnt signalling, transcription factor (Tcf3) activates the downstream genes that promote pluripotency maintenance by collaborating with the pivotal transcription factors Otc3/4 (Fig. 2), Sox2 and Nanog (Masui, 2010).

4. Derivation of human embryonic stem cells

There was a considerable delay between the derivation of mouse ES cells (1981) and the derivation of human ES cells in 1998 (Thomson et al., 1998). This delay was primarily due to species-specific ES cell differences and suboptimal human embryo culture media. In fact the first study to describe the isolation of human ICM cells was published by Bongso et al. (Bongso et al., 1994), but subsequent culture in media supplemented with LIF and serum resulted only in differentiation, not in the derivation of stable pluripotent cell lines. Human ES (hES) cells can be characterized by their immortality, expression of telomerase expression, pluripotentiality, ability to form teratomas, and maintenance of a stable karyotype and, even after prolonged undifferentiated proliferation, maintain the development potential to contribute to advanced derivatives of all three germ layers, even after clonal derivation (Amit et al., 2000). For obvious ethical reasons, experiments involving blastocyst injections and ectopic grafting in adult hosts cannot be performed in the human. Human ES cells have been derived from morula, later blastocyst embryos (Stojkovic et al., 2004; Strelchenko et al., 2004), single blastomeres (Klimanskaya et al., 2006), and parthenogenetic embryos (Lin et al., 2007). Previous reports suggest that the success rate in deriving hES cell lines is highly dependent on the quality of recovered blastocysts, isolation condition used and technical expertise (Pera et al., 2000; Mitalipova et al., 2003).

ES cell lines are usually derived by immunosurgery. In this process the trophoblast layer of the blastocyst is selectively removed, and the intact inner cell mass is further cultured on MEFs (Amit and Itskovitz-Eldor, 2002). Although the cloning efficiency of the hES cells was relatively poor, a several fold increase was observed when serum-free medium supplemented with basic fibroblast growth factor (bFGF) was used (Amit et al., 2000).

5. Maintenance of human embryonic stem cells

Mechanical and enzymatic transfer methods are used to maintain hES cell lines (Oh et al., 2005). The mechanical transfer method is laborious and time-consuming, although remains an efficient technique for the transfer of undifferentiated hES cells and results in similar clump sizes. The enzymatic transfer method is used when the bulk production of cells are required for various experiments and results in the more rapid growth and larger production of hES cells. However, the cell clumps vary in size, and there is a higher probability that both differentiated and undifferentiated cell will be transferred. In the case of passaging more differentiated colonies, a combination of both methods allows mass production of hES cells by excluding differentiated colonies from passage by manual selection prior to enzyme treatment.
Another limiting factor relating to cell culture systems is that hES cells still require the presence of feeder layer. In fact, feeder-free system for hES cell culture is required if hES cell cultures are to become clinical-grade, since the use of animal feeders and/or ingredients for growth of hES cells limits the large-scale culture and medical applicability of hES cells. At present, feeder-free systems are not optimal for the derivation and growth of clinical-grade hES cell lines since the presence of animal ingredient carries the potential risk for the cross-transfer of different infectious agents. In fact, it has been reported that hES cells embryoid body can incorporate the N-glycolyneuraminic acid (Neu5Gc) from MEFs or from conditioned medium, which resulted in an immune response (Martin et al., 2005).

The first attempt to produced feeder-free cultures of hES cells was reported by Xu et al. (Xu et al., 2001). They propagated hES cells using Matrigel, an animal based extracellular matrix (ECM) preparation, or laminin substrates in medium conditioned by MEFs. This system enabled the long term propagation of the stem cell phenotype, with strong suppression of spontaneous differentiation even at high passages (Carpenter et al., 2001).

In 2005, Prowse et al. identified 102 proteins from conditioned medium of human neonatal fibroblasts which provide invaluable information regarding the factors that may help maintain hES cells (Prowse et al., 2005).

The growth factor, ActivinA, paracrinely secreted by MEFs, is capable of supporting the growth of hES cells on laminin coated dishes for more 20 passages without the need for feeder layers (Beattie et al., 2005). Sato et al. (2004) suggest that Wnt signalling modulation can help to support the growth of hES cells cultures short-term and maintain their capacity to express some stem cell markers in the absence of a feeder cell layer (Sato et al., 2004). Another study demonstrated that noggin (BMP antagonist) combined with high βFGF concentrations in medium support the long term proliferation of undifferentiated hES cells in the absence of feeder cells and/or conditioned medium. However in this case Matrigel coated dishes were used, but this represent a problem for potential medical application of hES cells because xenogenic pathogens can be transmitted through culture conditions (Wang et al., 2005; Xu et al., 2005).

Moreover, it has been reported that the combination of FGF2, TGFβ, LIF and a proprietary serum replacer can achieve serum-free, feeder-free maintenance of hES cells when cultured on fibronectin ECM (Amit et al., 2004).

The establishment of feeder-free system for the culture of hES cells is critical for genetic manipulation. In fact, homologous recombination could be used as a tool for the repair of specific gene defects in stem cell lines derived from patients suffering disease.

6. Comparison between human and mouse ES cells

Many of the differences between mouse and human ES cells are only beginning to be elucidated, yet it has already been demonstrated that mouse and human ES cells differ in respect to cell surface markers, with human ES cells expressing the stage specific antigens SSEA-3 and SSEA-4, the glycoproteins TRA-1-60 and TRA-1-81, and GCTM-2, none of which are detected in the mouse. In contrast, mouse ES cells express SSEA-1, which remain undetected within human ES cultures. Moreover human ES cells are insensitive to the differentiation suppressing effects of LIF pathway (Thomson et al., 1998; Reubinoff et al., 2000). However, there remain many similarities between human and murine ES cell populations. ES cells are derived from both species using very similar protocols, and same aspects of their propagation, such as the ability of MEFs to support their growth in an undifferentiated state remain almost identical (Fig. 3). Furthermore, human and mouse ES cells possess similar
properties of spontaneous differentiation and expression of the pluripotent-associated transcription factor Oct-4.

![Mouse embryonic stem cell](image1) ![Human embryonic stem cell](image2)

Fig. 3. Phase contrast microscopy images of mouse (A) and human (B) embryonic stem cells on mouse embryonic fibroblast

7. Differentiation of mouse embryonic stem cells *in vitro*

In the absence of feeder cells and anti-differentiating agents such as LIF, mouse ES cells spontaneously differentiate and, under appropriate conditions, generate progeny consisting of derivatives of the three embryonic germ layer: mesoderm, endoderm, and ectoderm (Keller, 1995; Smith, 2001). Mesoderm derived lineages include the hematopoietic, vascular, and cardiac. Endoderm derivatives include pancreatic β cell and hepatocytes. Ectoderm differentiation of mouse ES cells is well established, as numerous studies have documented and characterized neuroectoderm commitment and neural differentiation. Three general approaches are used to initiate ES cell differentiation. With the first method, the hanging drop method (Fig. 4), ES cells are allowed to aggregate and form three dimensional colonies known as embryoid bodies (EBs) (Doetschman et al., 1985; Keller, 1995). In the second method, ES cells are cultured directly on stromal cells, and differentiation takes place in contact with these cells (Nakano et al., 1994). The third protocol involves differentiating ES cells in a monolayer on extracellular matrix proteins (Nishikawa et al., 1998) or in presence of specific differentiation medium (Takahashi et al., 2003; Fico et al., 2008).

7.1 Cardiac differentiation

The development of the cardiac lineage in ES cell differentiation cultures is easily detected by the appearance of areas of contracting cells that display characteristics of cardiomyocytes. Development of the cardiomyocyte lineage progresses through distinct stages that are similar to development of the lineage *in vivo*. An ordered pattern of expression of cardiac genes is observed in the differentiation cultures, with expression of the transcription factors *gata-4* and *nkx2.5* that are required for lineage development preceding the expression of genes such as *atrial natriuretic protein (ANP)*, *myosin light chain (MLC)-2v*, *α-
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myosin heavy chain (α-MHC), β-myosin heavy chain (β-MHC), and connexin 43 that are indicative of distinct maturation stages within the developing organ in vivo (Hescheler et al., 1997; Boheler et al., 2002). Several different studies have begun to investigate the mechanisms regulating the development of the cardiac lineage in ES cell differentiation cultures. It has been demonstrated that the EGF-CFC factor Cripto, known to be essential for development in vivo (Ding et al., 1998; Xu et al., 1999), plays a pivotal role in differentiation of ES cells to the cardiac lineage, in fact, Cripto−/− ES cells display a deficiency in generating cardiomyocytes (Parisi et al., 2003). Notch signaling also plays a role in cardiac development from ES cells (Schroeder et al., 2003), in fact ES cells lacking a downstream signalling molecule of all Notch (Jk) generate more cardiac cells than wild type ES cells (Keller, 2005). However, in this case, inhibition of the pathway appears to be important for cardiac differentiation. Other factors, including BMP2 and FGF2 (Kawai et al., 2004) as well as nitric oxide (Kanno et al., 2004) and ascorbic acid (Takahashi et al., 2003), have been shown to promote or improve cardiomyocyte differentiation in ES cell cultures.

Fig. 4. Schematic representation of method used to form embryoid bodies. This method is generally used to induce ES cells differentiation into cardiomyocytes or, adding retinoic acid, into neurons. MF-20 specific marker of cardiac cells, bIIITubulin (bIIITub) specific neural marker, DAPI nuclear marker

7.2 Primitive and definitive hematopoiesis
ES cells undergo hematopoietic differentiation in optimized culture conditions following serum induction (Keller, 1995). Gene expression and progenitor cell analysis revealed that
the differentiation program in these cultures closely parallels that in the early embryo, progressing through a primitive streak stage, to mesoderm, and subsequently to a yolk sac-like hematopoietic program. Detailed analysis of these early stages led to the identification of the hemangioblast, a progenitor that displays hematopoietic and vascular potential (Choi et al., 1998). After the hemangioblast appears, primitive erythroid progenitors develop in ES cells cultures, establishing the primitive erythropoiesis phase of hematopoiesis. In addition to primitive erythrocytes, other progenitors including those of the macrophage, definitive erythroid, megakaryocyte, and mast cell lineages develop in the differentiation cultures with a kinetic pattern similar to that observed in the yolk sac (Murry and Keller, 2008).

However, despite extensive efforts, to induce the formation of transplantable hematopoietic stem cells (HSCs) the development of HSCs from ESCs remains a challenge, which may reflect the complexities of embryonic hematopoietic development where different hematopoietic programs are generated at different times from different embryonic sites (Murry and Keller, 2008).

7.3 Endoderm differentiation

The generation of endoderm derivatives, in particular pancreatic β-cells and hepatocytes, has become the focus of many investigators in the field of ES cell biology. The interest in the efficient and reproducible development of these cell types derives from their clinical potential for the treatment of Type I diabetes and liver disease, respectively (Keller, 2005). Several genes used as markers of definitive endoderm (Foxa2, Gata4, and Sox17) (Arceci et al., 1993; Monaghan et al., 1993; Sasaki and Hogan, 1993; Laverriere et al., 1994; Kanai-Azuma et al., 2002), early liver (a-fetoprotein and albumin) (Dziadek and Adamson, 1978; Meehan et al., 1984; Sellem et al., 1984), and early pancreas (Pdx1 and insulin) (McGrath and Palis, 1997) development are also expressed by visceral endoderm, a population of extraembryonic endoderm. Given the overlapping expression patterns, it can be difficult to distinguish definitive and extraembryonic endoderm in the ES cell differentiation cultures. Another problem encountered in endoderm differentiation from ES cells is the lack of specific inducers of this lineage.

It has been investigated the potential of ES cells to differentiate into endoderm derivatives and developed two different protocols that promote the generation of these cell types (Kubo et al., 2004). The first is a restricted exposure of the EBs to serum followed by a period of serum-free culture, and the second is induction with Activin A in the absence of serum. Endoderm development was quantified based on the proportion of cells that expressed Foxa2, a transcription factor found in the earliest stages of definitive endoderm development (Monaghan et al., 1993; Sasaki and Hogan, 1993). All of the Foxa2+ cells that developed in these cultures also expressed the primitive streak marker brachyury, a gene that is not expressed in visceral endoderm. This observation strongly suggests that the Foxa2+ cells represented definitive endoderm. Based on the number of Foxa2+ cells, the Activin A protocol was found to be the most efficient as >50% of the total population in these cultures expressed this protein, in fact, low level of Activin A promote a mesoderm fate, and high levels of Activin A induced the formation of endoderm cells (Green et al., 1992; Hudson et al., 1997).

In 2009 Borowiak et al. identified two potent small molecules, IDE1 and IDE2, that can direct mouse ES cell differentiation such that 70%–80% of cells are endoderm cells. This efficiency of induction compares favorably with published protocols employing TGF-β family members, e.g., Activin A or Nodal, which produce about 45% endoderm. The application of small molecules to differentiate mouse and human ES cells into endoderm represents a step
toward achieving a reproducible and efficient production of desired ES cell derivatives (Borowiak et al., 2009).

7.4 Neural differentiation
Several different protocols have evolved to promote neuroectoderm differentiation. The various approaches include (1) treatment of serum-stimulated EBs with retinoic acid (Bain et al., 1995), (2) sequential culture of EBs in serum followed by serum-free medium (Okabe et al., 1996), (3) differentiation of ES cells as a monolayer in serum-free medium (Tropepe et al., 2001; Ying et al., 2003; Fico et al., 2008), and (4) differentiation of ES cells directly on stromal cells in the absence of serum (Kawasaki et al., 2000; Barberi et al., 2003). As with the mesoderm and endoderm lineages, development of the ectoderm lineages in the ES differentiation cultures appears to recapitulate their development in the early embryo (Barberi et al., 2003). In vitro it is possible to form the three major neural cell types: neurons, astrocytes and oligodendrocytes.

The protocols for differentiation to specific types of neurons have included the sequential combination of regulators that are known to play a role in the establishment of these lineages in the early embryo. For instance, midbrain dopaminergic neurons have been generated in the EB system by overexpression in the cells of the transcription factor nuclear-receptor-related factor1 (Nurr1), and the addition to the cultures of sonic hedgehog (SHH) and FGF8 (Kim et al., 2002). Nurr1, SHH, and FGF8 are required for the development of this class of neurons in the early embryo (Ye et al., 1998; Simon et al., 2003). Other studies have demonstrated the development of cholinergic, serotonergic, and GABAergic neurons in addition to dopaminergic neurons, when differentiated on MS5 stromal cells in the presence of different combinations of cytokines (Barberi et al., 2003). Using the coculture approach together with the appropriate signaling molecules and selection steps, cells that display many of the characteristics of motor neurons has been successfully generated (Wichterle et al., 2002).

When cultured at low density in serum-free medium in the presence of LIF, ES cells generate a population that has been called primitive neural stem cells (Tropepe et al., 2001). These cells have been characterized by their ability to generate neurosphere-like colonies composed of cells that express the neural precursor cell marker, nestin (Lendahl et al., 1990). When cultured on a matrigel substrate in the presence of low amounts of serum, cells within these colonies generated neurons, astrocytes, and oligodendrocytes. In 2008, Fico et al. established a one-step protocol that allowed differentiation of mouse ES cells into a highly enriched population of neuronal cells, simply by culturing them on gelatin-coated dishes in a chemically defined serum-free medium. This differentiation method is able to generate a wide range of neural subtypes and glial cells from mouse ES cells (Fico et al., 2008).

The ability to generate different types of neurons from ES cells has dramatically raised the interest in repair of nervous system disorders by cell replacement therapy.

8. Human embryonic stem cells differentiation

Human embryonic stem cells are characterized by their ability to proliferate in the undifferentiated state in culture for a prolonged period, and by their capacity to differentiate into derivatives of all three germ layers. A variety of studies have described in vitro spontaneous and directed differentiation of hES cells into different lineages: cardiomyocytes (Kehat et al., 2001; Xu et al., 2002), neurons and glia (Carpenter et al., 2001; Reubinoff et al.,

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2001), endothelial cells (Levenberg et al., 2002), hematopoietic precursors (Kaufman et al., 2001), trophoblast, and hepatocyte-like cells (Rambhatla et al., 2003). The most common method used for *in vitro* differentiation is to remove the hES cells from the feeder layer and culture in suspension in absence of MEFs. Following culturing in suspension, hES cells aggregate into EBs (Itskovitz-Eldor et al., 2000). The aggregation process itself triggers initial cell differentiation. It is thought that the EBs consist of derivatives of all three germ layers, which interact and cross-induce each other, resulting in complex differentiation into the various lineages. This process is considered to recapitulate early embryonic development from the blastocyst stage to the egg-cylinder stage.

### 8.1 Cardiac differentiation

In order to generate a cardiomyocyte-differentiating system from the hES cells, small clumps of 3–20 cells were grown in suspension for 8 days (Amit et al., 2000). The EBs were then plated on gelatin-coated culture dishes and observed microscopically for the appearance of spontaneous contraction. Rhythmically contracting areas appeared at 6 to 12 days after plating. Cells isolated from the beating areas expressed cardiac-specific structural genes, such as cardiac troponin I and brachyury (T), atrial natriuretic peptide (ANP), atrial and ventricular myosin light chains (MLCs). Immunostaining studies demonstrated the presence of the cardiac-specific sarcomeric proteins myosin heavy chain, α-actinin, desmin, and cardiac troponin I, as well as ANP (Kehat et al., 2001).

Cardiomyocyte differentiation can be enhanced in the mouse ES cell system following the addition of differentiation factors including, dimethyl sulfoxide (DMSO), retinoic acid (RA), and small molecules. Addition of the demethylating agent 5-aza-2’-deoxycytidine to EB cultures has also been shown to be effective for mouse ES cell and human ES cell differentiation into cardiomyocytes. In contrast, RA in hES cells did not induce a higher proportion of cardiomyocytes *in vitro* (Schuldiner et al., 2000). An alternative method for deriving cardiomyocytes has been achieved following the coculture of pluripotent hES cell lines with END-2 cells (visceral-endoderm-like cell lines) (Mummery et al., 2003).

### 8.2 Hematopoietic differentiation

Several studies have documented hematopoietic development of hES cells using different induction schemes (Murry and Keller, 2008). As observed in the mouse system, the predominant population generated during the first 7–10 days of hES cell differentiation is primitive erythroid progenitors, indicating that the equivalent of yolk-sac hematopoiesis develops first in these cultures (Zambidis et al., 2005; Kennedy et al., 2007). As observed with mouse ES cell and the mouse embryo, the onset of hematopoiesis in hES cell cultures is marked by development of the hemangioblast between days 2 and 4 of differentiation, prior to establishment of the primitive erythroid lineage (Kennedy et al., 2007; Lu et al., 2007; Davis et al., 2008).

### 8.3 Neural differentiation

In 2001, Reubinoff and Zhang highlighted the potential of hES cells to generated neural cells (Reubinoff et al., 2001; Zhang et al., 2001). Zhang et al. have combined the techniques which were initially developed for the neural differentiation of mouse ES cells and adapted these to produce human neural stem cells. This occurs via a successive stepwise approach, which consists of inducing the formation of EBs and from these generating neural rosettes, which
are proliferating structures that mimic neural tube formation. Rosettes are subsequently harvested by selective dissociation and are cultured as free-floating aggregates of neural precursors, capable of generating neurons and glia (Zhang et al., 2001). Reubinoff demonstrated that neural differentiation was induced by overgrowth of undifferentiated ES cells. Maintaining hES cells in culture without passage or replenishing feeder cells led to spontaneous neural differentiation within a heterogeneous population of hES cell progeny. Individual clusters of presumptive neural progenitors were identified by phase contrast microscopy and manually transferred onto uncoated dishes. Following culture in defined medium supplemented with βFGF and epidermal growth factor (EGF), these cells formed aggregates highly enriched with neural precursor cells. After withdrawal of βFGF and EGF, downregulation of nestin and mash-1 is followed by upregulated expression of neuron-specific NFM, synaptophysin, Nurr1, and tyrosine hydroxylase (TH) genes. A decreased formation of nestin-positive cells is assimilated with an increased number of neuronal cells expressing neuron-specific protein. Mature neuronal cells are evidenced by the production of neurotransmitters such as dopamine, serotonin, GABA, and glutamate. These results suggest that in presence of neuronal differentiation factors, such as retinoic acid, FGF4, FGF8, or βFGF, hES-derived cells, led to the enrichment of cholinergic, serotonergic, dopaminergic and GABAergic neurons, respectively (Okabe et al., 1996; Lee et al., 2000; Rolletschek et al., 2001; Barberi et al., 2003).

Li et al. (2005) differentiated hES cells into spinal motoneurons using retinoic acid and in the presence of SHH (Li et al., 2005).

### 8.4 Pancreatic β -islet cells

1–3% of cells within 60–70% of human EBs produced from hES cells have been observed to stain positively for insulin (Assady et al., 2001). A modification of Lumelskey and colleagues (2001) method resulted in the production of insulin-secreting cells derived from hES cells (Lumelsky et al., 2001). This was achieved following an additional step of culture including, a lowering of the glucose concentration in the medium, removal of βFGF and addition of nicotinamide. Dissociating the cells and growing them in suspension resulted in the formation of clusters, which secreted higher levels of insulin than their in vivo counterparts and could be maintained in vitro. These cells expressed pancreatic genes and following immunofluorescence and in situ hybridization studies, it was confirmed that a high percentage of insulin-expressing cells were located within these cell clusters (Segev et al., 2004).

### 9. A new age for ES cells: induced pluripotent stem cells

Takahashi and Yamanaka recently achieved a significant breakthrough in reprogramming somatic cells back to an ES like state (Takahashi and Yamanaka, 2006). They 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 followed by selection for activation of the Oct4 target gene Fbx15. Cells that had activated Fbx15 were designated with a coined expression “induced pluripotent stem” (iPS) cells. These cells were shown to be pluripotent by their ability to form teratomas although they were unable to generate live chimeras. In subsequent experiments when activation of the endogenous Oct4 or Nanog genes was used as a more stringent selection criterion for
pluripotency, the resulting Oct4-iPS or Nanog-iPS cells, in contrast to Fbx15-iPS cells, were fully reprogrammed to a pluripotent ES cell state by molecular and biological criteria (Maherali et al., 2007; Wernig et al., 2007). Shortly after the reprogramming of mouse cells had been achieved the generation of iPS cells from human fibroblasts was reported (Takahashi et al., 2007; Yu et al., 2007).

While genetic experiments have established that Oct4 and Sox2 are essential for pluripotency (Chambers and Smith, 2004), the role of the two oncogenes, c-myc and Klf4, in reprogramming is less clear. Some of these oncogenes may, in fact, be dispensable for reprogramming as both mouse and human iPS cells have been obtained in the absence of c-myc transduction, although with low efficiency (Nakagawa et al., 2008; Wernig et al., 2008).

One of the promises of patient-specific ES cells is the potential for customized therapy of diseases. Previous studies have shown that disease-specific ES cells produced by nuclear cloning in combination with gene correction can be used to correct an immunologic disorder in a proof-of-principle experiment in mice (Rideout et al., 2002). In a similar approach, by using a humanized sickle cell anemia mouse model, it has been shown that mice can be rescued after transplantation with hematopoietic progenitors obtained in vitro from autologous iPS cells (Hanna et al., 2007). Finally, it has been shown that iPS cells can be efficiently differentiated into neural precursor cells giving rise to neuronal and glial cell types in culture. Neural precursors derived from iPS cell were able to improve behaviour in a rat model of Parkinson’s disease upon transplantation into the adult brain demonstrating the therapeutic potential of directly reprogrammed fibroblasts for neuronal cell replacement in an animal model (Wernig et al., 2008; Jaenisch, 2009).

10. Conclusion

Embryonic stem cells represent a powerful tool for future regenerative medicine due to their capacity of self-renewal and pluripotency. Studies in animal models have shown that transplantation of fetal stem cell, ES cells, or pluripotent stem cell derivatives can successfully treat many chronic diseases, such as Parkinson’s disease, diabetes, traumatic spinal cord injury, Purkinje cell degeneration, Duchenne’s muscular dystrophy, liver or heart failure, and osteogenesis imperfecta (Zhang et al., 1996; Horwitz et al., 1999; McDonald et al., 1999; Kobayashi et al., 2000; Li et al., 2000; Soria et al., 2000; Kim et al., 2002).

Almost every day there are reports in the media of new stem cell therapies. There is no doubt that stem cells have the potential to treat many human afflictions, including ageing, cancer, diabetes, blindness and neurodegeneration. In January 2009, the US Food and Drug Administration approved the first clinical trial involving human ES cells, just over 10 years after they were first isolated. In this trial, the safety of ES cell-derived oligodendrocytes in repair of spinal cord injury will be evaluated. Nevertheless, one of the attractions of transplanting iPS cells is that the patient’s own cells can be used, obviating the need for immunosuppression (Watt and Driskell, 2010).

Adult tissue stem cells, ES cells and iPS cells can all be used to screen for compounds that stimulate selfrenewal or promote specific differentiation programmes. Finding drugs that selectively target cancer stem cells offers the potential to develop cancer treatments that are not only more effective, but also cause less collateral damage to the patient’s normal tissues than drugs currently in use (Watt and Driskell, 2010).
11. References

Abbondanzo, S.J.; Gadi, I. & Stewart, C.L. (1993) Derivation of embryonic stem cell lines. *Methods Enzymol.*, 225, 1993) 803-23, ISSN: 0076-6879

Amit, M.; Carpenter, M.K.; Inokuma, M.S.; Chiu, C.P.; Harris, C.P.; Waknitz, M.A.; Itskovitz-Eldor, J. & Thomson, J.A. (2000) Clonally derived human embryonic stem cell lines maintain pluripotency and proliferative potential for prolonged periods of culture. *Dev Biol*, 227, 2, (Nov 2000) 271-8, ISSN: 0012-1606

Amit, M. & Itskovitz-Eldor, J. (2002) Derivation and spontaneous differentiation of human embryonic stem cells. *J Anat*, 200, Pt 3, (Mar 2002) 225-32, ISSN: 0021-8782

Amit, M.; Shariki, C.; Margulets, V. & Itskovitz-Eldor, J. (2004) Feeder layer- and serum-free culture of human embryonic stem cells. *Biol Reprod*, 70, 3, (Mar 2004) 837-45, ISSN: 0006-3363

Arceci, R.J.; King, A.A.; Simon, M.C.; Orkin, S.H. & Wilson, D.B. (1993) Mouse GATA-4: a retinoic acid-inducible GATA-binding transcription factor expressed in endodermally derived tissues and heart. *Mol Cell Biol*, 13, 4, (Apr 1993) 2235-46, ISSN: 0270-7306

Assady, S.; Maor, G.; Amit, M.; Itskovitz-Eldor, J.; Skorecki, K.L. & Tzukerman, M. (2001) Insulin production by human embryonic stem cells. *Diabetes*, 50, 8, (Aug 2001) 1691-7, ISSN: 0012-1797

Bain, G.; Kitchens, D.; Yao, M.; Huettner, J.E. & Gottlieb, D.I. (1995) Embryonic stem cells express neuronal properties in vitro. *Dev Biol*, 168, 2, (Apr 1995) 342-57, ISSN: 0012-1606

Barberi, T.; Klivenyi, P.; Calingasan, N.Y.; Lee, H.; Kawamata, H.; Loonam, K.; Perrier, A.L.; Bruses, J.; Rubio, M.E.; Topf, N.; Tabar, V.; Harrison, N.L.; Beal, M.F.; Moore, M.A. & Studer, L. (2003) Neural subtype specification of fertilization and nuclear transfer embryonic stem cells and application in parkinsonian mice. *Nat Biotechnol*, 21, 10, (Oct 2003) 1200-7, ISSN: 1087-0156

Beattie, G.M.; Lopez, A.D.; Bucay, N.; Hinton, A.; Firpo, M.T.; King, C.C. & Hayek, A. (2005) Activin A maintains pluripotency of human embryonic stem cells in the absence of feeder layers. *Stem Cells*, 23, 4, (Apr 2005) 489-95, ISSN: 1066-5099

Boheler, K.R.; Czyz, J.; Tweedie, D.; Yang, H.T.; Anisimov, S.V. & Wobus, A.M. (2002) Differentiation of pluripotent embryonic stem cells into cardiomyocytes. *Circ Res*, 91, 3, (Aug 2002) 189-201, ISSN: 0009-7330

Bongso, A.; Fong, C.Y.; Ng, S.C. & Ratnam, S. (1994) Isolation and culture of inner cell mass cells from human blastocysts. *Hum Reprod*, 9, 11, (Nov 1994) 2110-7, ISSN: 0268-1161

Borowiak, M.; Maehr, R.; Chen, S.; Chen, A.E.; Tang, W.; Fox, J.L.; Schreiber, S.L. & Melton, D.A. (2009) Small molecules efficiently direct endodermal differentiation of mouse and human embryonic stem cells. *Cell Stem Cell*, 4, 4, (Apr 3 2009) 348-58, ISSN: 1934-5909

Bradley, A.; Evans, M.; Kaufman, M.H. & Robertson, E. (1984) Formation of germ-line chimaeras from embryo-derived teratocarcinoma cell lines. *Nature*, 309, 5965, (May 1984) 255-6, ISSN: 0028-0836

Bryja, V.; Bonilla, S. & Arenas, E. (2006a) Derivation of mouse embryonic stem cells. *Nat Protoc*, 1, 4, 2006a) 2082-7, ISSN: 1754-2189
Bryja, V.; Bonilla, S.; Cajanek, L.; Parish, C.L.; Schwartz, C.M.; Luo, Y.; Rao, M.S. & Arenas, E. (2006b) An efficient method for the derivation of mouse embryonic stem cells. *Stem Cells*, 24, 4, (Apr 2006b) 844-9, ISSN: 1066-5099

Burdon, T.; Smith, A. & Savatier, P. (2002) Signalling, cell cycle and pluripotency in embryonic stem cells. *Trends Cell Biol*, 12, 9, (Sep 2002) 432-8, ISSN: 0962-8924

Carpenter, M.K.; Inokuma, M.S.; Denham, J.; Mujtaba, T.; Chiu, C.P. & Rao, M.S. (2001) Enrichment of neurons and neural precursors from human embryonic stem cells. *Exp Neurol*, 172, 2, (Dec 2001) 383-97, ISSN: 0014-4886

Chambers, I. & Smith, A. (2004) Self-renewal of teratocarcinoma and embryonic stem cells. *Oncogene*, 23, 43, (Sep 2004) 7150-60, ISSN: 0950-9232

Choi, K.; Kennedy, M.; Kazarov, A.; Papadimitriou, J.C. & Keller, G. (1998) A common precursor for hematopoietic and endothelial cells. *Development*, 125, 4, (Feb 1998) 725-32, ISSN: 0950-1991

Chung, Y.; Klimanskaya, I.; Becker, S.; Marh, J.; Lu, S.J.; Johnson, J.; Meisner, L. & Lanza, R. (2006) Embryonic and extraembryonic stem cell lines derived from single mouse blastomeres. *Nature*, 439, 7073, (Jan 2006) 216-9, ISSN: 0028-0836

Cibelli, J.B.; Stice, S.L.; Golueke, P.J.; Kane, J.J.; Jerry, J.; Blackwell, C.; Ponce de Leon, F.A. & Robl, J.M. (1998) Transgenic bovine chimeric offspring produced from somatic cell-derived stem-like cells. *Nat Biotechnol*, 16, 7, (Jul 1998) 642-6, ISSN: 1087-0156

Davis, R.P.; Ng, E.S.; Costa, M.; Mossman, A.K.; Sourris, K.; Elefanty, A.G. & Stanley, E.G. (2008) Targeting a GFP reporter gene to the MIXL1 locus of human embryonic stem cells identifies human primitive streak-like cells and enables isolation of primitive hematopoietic precursors. *Blood*, 111, 4, (Feb 15 2008) 1876-84, ISSN: 0006-4971

de Haan, G. (2002) Hematopoietic stem cells: self-renewing or aging? *Cells Tissues Organs*, 171, 1, 2002) 27-37, ISSN: 1422-6405

Ding, J.; Yang, L.; Yan, Y.T.; Chen, A.; Desai, N.; Wynshaw-Boris, A. & Shen, M.M. (1998) Cripto is required for correct orientation of the anterior-posterior axis in the mouse embryo. *Nature*, 395, 6703, (Oct 1998) 702-7, ISSN: 0028-0836

Doetschman, T.; Williams, P. & Maeda, N. (1988) Establishment of hamster blastocyst-derived embryonic stem (ES) cells. *Dev Biol*, 127, 1, (May 1988) 224-7, ISSN: 0012-1606

Doetschman, T.C.; Eistetter, H.; Katz, M.; Schmidt, W. & Kemler, R. (1985) The in vitro development of blastocyst-derived embryonic stem cell lines: formation of visceral yolk sac, blood islands and myocardium. *J Embryol Exp Morphol*, 87, (Jun 1985) 27-45, ISSN: 0022-0752

Dziadek, M. & Adamson, E. (1978) Localization and synthesis of alphafoetoprotein in post-implantation mouse embryos. *J Embryol Exp Morphol*, 43, (Feb 1978) 289-313, ISSN: 0022-0752

Evans, M.J. & Kaufman, M.H. (1981) Establishment in culture of pluripotential cells from mouse embryos. *Nature*, 292, 5819, (Jul 1981) 154-6, ISSN: 0028-0836

Fico, A.; Manganelli, G.; Simeone, M.; Guido, S.; Minchiotti, G. & Filosa, S. (2008) High-throughput screening-compatible single-step protocol to differentiate embryonic stem cells in neurons. *Stem Cells Dev*, 17, 3, (Jun 2008) 573-84, ISSN: 1547-3287

Green, J.B.; New, H.V. & Smith, J.C. (1992) Responses of embryonic Xenopus cells to activin and FGF are separated by multiple dose thresholds and correspond to distinct axes of the mesoderm. *Cell*, 71, 5, (Nov 1992) 731-9, ISSN: 0092-8674
Hanna, J.; Wernig, M.; Markoulaki, S.; Sun, C.W.; Meissner, A.; Cassady, J.P.; Beard, C.; Brambrink, T.; Wu, L.C.; Townes, T.M. & Jaenisch, R. (2007) Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. Science, 318, 5858, (Dec 2007) 1920-3, ISSN: 0036-8075

Hescheler, J.; Fleischmann, B.K.; Lentini, S.; Maltsev, V.A.; Rohwedel, J.; Wobus, A.M. & Addicks, K. (1997) Embryonic stem cells: a model to study structural and functional properties in cardiomyogenesis. Cardiovasc Res, 36, 2, (Nov 1997) 149-62, ISSN: 0008-6363

Hogan, B.; Beddington, R.; Costantini, F. & Lacy, E. (1994) Manipulating the Mouse Embryo: A Laboratory Manual (Second Edition). Cold Spring Harbor Laboratory Press, ISBN: 0-87969-384-3, New York, USA

Hong, Y.; Winkler, C. & Schartl, M. (1998) Production of medakafish chimeras from a stable embryonic stem cell line. Proc Natl Acad Sci U S A, 95, 7, (Mar 1998) 3679-84, ISSN: 0027-8424

Horwitz, E.M.; Prockop, D.J.; Fitzpatrick, L.A.; Koo, W.W.; Gordon, P.L.; Neel, M.; Sussman, M.; Orchard, P.; Marx, J.C.; Pyeritz, R.E. & Brenner, M.K. (1999) Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta. Nat Med, 5, 3, (Mar 1999) 309-13, ISSN: 1078-8956

Hudson, C.; Clements, D.; Friday, R.V.; Stott, D. & Woodland, H.R. (1997) Sox17alpha and -beta mediate endoderm formation in Xenopus. Cell, 91, 3, (Oct 1997) 397-405, ISSN: 0092-8674

Itskovitz-Eldor, J.; Schuldiner, M.; Karsenti, D.; Eden, A.; Yanuka, O.; Amit, M.; Soreq, H. & Benvenisty, N. (2000) Differentiation of human embryonic stem cells into embryoid bodies compromising the three embryonic germ layers. Mol Med, 6, 2, (Feb 2000) 88-95, ISSN: 1076-1551

Jaenisch, R. (2009) Stem cells, pluripotency and nuclear reprogramming. J Thromb Haemost, 7 Suppl 1, (Jul 2009) 21-3, ISSN: 1538-7933

Kanai-Azuma, M.; Kanai, Y.; Gad, J.M.; Tajima, Y.; Taya, C.; Kurohmaru, M.; Sanai, Y.; Yonekawa, H.; Yazaki, K.; Tam, P.P. & Hayashi, Y. (2002) Depletion of definitive gut endoderm in Sox17-null mutant mice. Development, 129, 10, (May 2002) 2367-79, ISSN: 0950-1991

Kanno, S.; Kim, P.K.; Sallam, K.; Lei, J.; Billiar, T.R. & Shears, L.L., 2nd (2004) Nitric oxide facilitates cardiomyogenesis in mouse embryonic stem cells. Proc Natl Acad Sci U S A, 101, 33, (Aug 2004) 12277-81, ISSN: 0027-8424

Kaufman, D.S.; Hanson, E.T.; Lewis, R.L.; Auerbach, R. & Thomson, J.A. (2001) Hematopoietic colony-forming cells derived from human embryonic stem cells. Proc Natl Acad Sci U S A, 98, 19, (Sep 2001) 10716-21, ISSN: 0027-8424

Kawai, T.; Takahashi, T.; Esaki, M.; Ushikoshi, H.; Nagano, S.; Fujiwara, H. & Kosai, K. (2004) Efficient cardiomyogenic differentiation of embryonic stem cell by fibroblast growth factor 2 and bone morphogenetic protein 2. Circ J, 68, 7, (Jul 2004) 691-702, ISSN: 1346-9843

Kawasaki, H.; Mizuseki, K.; Nishikawa, S.; Kaneko, S.; Kuwana, Y.; Nakanishi, S.; Nishikawa, S.I. & Sasai, Y. (2000) Induction of midbrain dopaminergic neurons from ES cells by stromal cell-derived inducing activity. Neuron, 28, 1, (Oct 2000) 31-40, ISSN: 0896-6273
Kehat, I.; Kenyagin-Karsenti, D.; Snir, M.; Segev, H.; Amit, M.; Gepstein, A.; Livne, E.; Binah, O.; Itskovitz-Eldor, J. & Gepstein, L. (2001) Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. *J Clin Invest*, 108, 3, (Aug 2001) 407-14, ISSN: 0021-9738

Keller, G. (2005) Embryonic stem cell differentiation: emergence of a new era in biology and medicine. *Genes Dev*, 19, 10, (May 2005) 1129-55, ISSN: 0890-9369

Keller, G.M. (1995) *In vitro* differentiation of embryonic stem cells. *Curr Opin Cell Biol*, 7, 6, (Dec 1995) 862-9, ISSN: 0955-0674

Kennedy, M.; D’Souza, S.L.; Lynch-Kattman, M.; Schwantz, S. & Keller, G. (2007) Development of the hemangioblast defines the onset of hematopoiesis in human ES cell differentiation cultures. *Blood*, 109, 7, (Apr 1 2007) 2679-87, ISSN: 0006-4971

Kim, J.H.; Auerbach, J.M.; Rodriguez-Gomez, J.A.; Velasco, I.; Gavin, D.; Lumelsky, N.; Lee, S.H.; Nguyen, J.; Sanchez-Pernaute, R.; Bankiewicz, K. & McKay, R. (2002) Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease. *Nature*, 418, 6893, (Jul 2002) 50-6, ISSN: 0028-0836

Klimanskaya, I.; Chung, Y.; Becker, S.; Lu, S.J. & Lanza, R. (2006) Human embryonic stem cell lines derived from single blastomeres. *Nature*, 444, 7118, (Nov 2006) 481-5, ISSN: 0028-0836

Kobayashi, N.; Miyazaki, M.; Fukaya, K.; Inoue, Y.; Sakaguchi, M.; Uemura, T.; Noguchi, H.; Kondo, A.; Tanaka, N. & Namba, M. (2000) Transplantation of highly differentiated immortalized human hepatocytes to treat acute liver failure. *Transplantation*, 69, 2, (Jan 2000) 202-7, ISSN: 0041-1337

Kubo, A.; Shinozaki, K.; Shannon, J.M.; Kouskoff, V.; Kennedy, M.; Woo, S.; Fehling, H.J. & Keller, G. (2004) Development of definitive endoderm from embryonic stem cells in culture. *Development*, 131, 7, (Apr 2004) 1651-62, ISSN: 0950-1991

Laverriere, A.C.; MacNeill, C.; Mueller, C.; Poelmann, R.E.; Burch, J.B. & Evans, T. (1994) GATA-4/5/6, a subfamily of three transcription factors transcribed in developing heart and gut. *J Biol Chem*, 269, 37, (Sep 1994) 23177-84, ISSN: 0021-9258

Ledermann, B. & Burki, K. (1991) Establishment of a germ-line competent C57BL/6 embryonic stem cell line. *Exp Cell Res*, 197, 2, (Dec 1991) 254-8, ISSN: 0014-4827

Lee, S.H.; Lumelsky, N.; Studer, L.; Auerbach, J.M. & McKay, R.D. (2000) Efficient generation of midbrain and hindbrain neurons from mouse embryonic stem cells. *Nat Biotechnol*, 18, 6, (Jun 2000) 675-9, ISSN: 1087-0156

Lendahl, U.; Zimmerman, L.B. & McKay, R.D. (1990) CNS stem cells express a new class of intermediate filament protein. *Cell*, 60, 4, (Feb 1990) 585-95, ISSN: 0092-8674

Levenberg, S.; Golub, J.S.; Amit, M.; Itskovitz-Eldor, J. & Langer, R. (2002) Endothelial cells derived from human embryonic stem cells. *Proc Natl Acad Sci U S A*, 99, 7, (Apr 2002) 4391-6, ISSN: 0027-8424

Li, M.; Zhang, D.; Hou, Y.; Jiao, L.; Zheng, X. & Wang, W.H. (2003) Isolation and culture of embryonic stem cells from porcine blastocysts. *Mol Reprod Dev*, 65, 4, (Aug 2003) 429-34, ISSN: 1040-452X

Li, R.K.; Weisel, R.D.; Mickle, D.A.; Jia, Z.Q.; Kim, E.J.; Sakai, T.; Tomita, S.; Schwartz, L.; Iwanochko, M.; Husain, M.; Cusimano, R.J.; Burns, R.J. & Yau, T.M. (2000) Autologous porcine heart cell transplantation improved heart function after a myocardial infarction. *J Thorac Cardiovasc Surg*, 119, 1, (Jan 2000) 62-8, ISSN: 0022-5223
Li, X.J.; Du, Z.W.; Zarnowska, E.D.; Pankratz, M.; Hansen, L.O.; Pearce, R.A. & Zhang, S.C. (2005) Specification of motoneurons from human embryonic stem cells. *Nat Biotechnol*, 23, 2, (Feb 2005) 215-21, ISSN: 1087-0156

Lin, G.; OuYang, Q.; Zhou, X.; Gu, Y.; Yuan, D.; Li, W.; Liu, G.; Liu, T. & Lu, G. (2007) A highly homozygous and parthenogenetic human embryonic stem cell line derived from a one-pronuclear oocyte following *in vitro* fertilization procedure. *Cell Res*, 17, 12, (Dec 2007) 999-1007, ISSN: 1001-0602

Lu, S.J.; Feng, Q.; Caballero, S.; Chen, Y.; Moore, M.A.; Grant, M.B. & Lanza, R. (2007) Generation of functional hemangioblasts from human embryonic stem cells. *Nat Methods*, 4, 6, (Jun 2007) 501-9, ISSN: 1548-7091

Lumelsky, N.; Blondel, O.; Laeng, P.; Velasco, I.; Ravin, R. & McKay, R. (2001) Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets. *Science*, 292, 5520, (May 2001) 1389-94, ISSN: 0036-8075

Maherali, N.; Sridharan, R.; Xie, W.; Utikal, J.; Eminli, S.; Arnold, K.; Stadtfeld, M.; Yachechko, R.; Tchieu, J.; Jaenisch, R.; Plath, K. & Hochedlinger, K. (2007) Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. *Cell Stem Cell*, 1, 1, (Jun 2007) 55-70, ISSN: 1934-5909

Martin, G.R. (1981) Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc Natl Acad Sci U S A*, 78, 12, (Dec 1981) 7634-8, ISSN: 0027-8424

Matsuda, T.; Nakamura, T.; Nakao, K.; Arai, T.; Katsuki, M.; Heike, T. & Yokota, T. (1999) STAT3 activation is sufficient to maintain an undifferentiated state of mouse embryonic stem cells. *Embo J*, 18, 15, (Aug 1999) 4261-9, ISSN: 0261-4189

McDonald, J.W.; Liu, X.Z.; Qu, Y.; Liu, S.; Mickey, S.K.; Turetsky, D.; Gottlieb, D.I. & Choi, D.W. (1999) Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord. *Nat Med*, 5, 12, (Dec 1999) 1410-2, ISSN: 1078-8956

McGrath, K.E. & Palis, J. (1997) Expression of homeobox genes, including an insulin promoting factor, in the murine yolk sac at the time of hematopoietic initiation. *Mol Reprod Dev*, 48, 2, (Oct 1997) 145-53, ISSN: 1040-452X

McWhir, J.; Schnieke, A.E.; Ansell, R.; Wallace, H.; Colman, A.; Scott, A.R. & Kind, A.J. (1996) Selective ablation of differentiated cells permits isolation of embryonic stem cell lines from murine embryos with a non-permissive genetic background. *Nat Genet*, 14, 2, (Oct 1996) 223-6, ISSN: 1061-4036

Meehan, R.R.; Barlow, D.P.; Hill, R.E.; Hogan, B.L. & Hastie, N.D. (1984) Pattern of serum protein gene expression in mouse visceral yolk sac and foetal liver. *Embo J*, 3, 8, (Aug 1984) 1881-5, ISSN: 0261-4189

Mitalipova, M.; Calhoun, J.; Shin, S.; Wininger, D.; Schulz, T.; Noggle, S.; Venable, A.; Lyons, I.; Robins, A. & Stice, S. (2003) Human embryonic stem cell lines derived from discarded embryos. *Stem Cells*, 21, 5, 2003) 521-6, ISSN: 1066-5099
Monaghan, A.P.; Kaestner, K.H.; Grau, E. & Schutz, G. (1993) Postimplantation expression patterns indicate a role for the mouse forkhead/HNF-3 alpha, beta and gamma genes in determination of the definitive endoderm, chordamesoderm and neuroectoderm. Development, 119, 3, (Nov 1993) 567-78, ISSN: 0950-1991

Mummery, C.; Ward-van Oostwaard, D.; Doevendans, P.; Spijker, R.; van den Brink, S.; Hassink, R.; van der Heyden, M.; Opthof, T.; Pera, M.; de la Riviere, A.B.; Passier, R. & Tertoolen, L. (2003) Differentiation of human embryonic stem cells to cardiomyocytes: role of coculture with visceral endoderm-like cells. Circulation, 107, 21, (Jun 2003) 2733-40, ISSN: 0009-7322

Murry, C.E. & Keller, G. (2008) Differentiation of embryonic stem cells to clinically relevant populations: lessons from embryonic development. Cell, 132, 4, (Feb 22 2008) 661-80, ISSN: 0092-8674

Nagy, A.; Gertsenstein, M.; Vintersten, K. & Behringer, R. (2003) Manipulating the Mouse Embryo: A Laboratory Manual (Third Edition). Cold Spring Harbor Laboratory Press, ISBN: 978-087969591-0, New York, USA

Nagy, A.; Gocza, E.; Diaz, E.M.; Prideaux, V.R.; Ivanyi, E.; Markkula, M. & Rossant, J. (1990) Embryonic stem cells alone are able to support fetal development in the mouse. Development, 110, 3, (Nov 1990) 815-21, ISSN: 0950-1991

Nakagawa, M.; Koyanagi, M.; Tanabe, K.; Takahashi, K.; Ichisaka, T.; Aoi, T.; Okita, K.; Mochiduki, Y.; Takizawa, N. & Yamanaka, S. (2008) Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. Nat Biotechnol, 26, 1, (Jan 2008) 101-6, ISSN: 1087-0156

Nakano, T.; Kodama, H. & Honjo, T. (1994) Generation of lymphohematopoietic cells from embryonic stem cells in culture. Science, 265, 5175, (Aug 1994) 1098-101, ISSN: 0036-8075

Nishikawa, S.I.; Nishikawa, S.; Hirashima, M.; Matsuyoshi, N. & Kodama, H. (1998) Progressive lineage analysis by cell sorting and culture identifies FLK1+VE-cadherin+ cells at a diverging point of endothelial and hemopoietic lineages. Development, 125, 9, (May 1998) 1747-57, ISSN: 0950-1991

Niwa, H.; Ogawa, K.; Shimosato, D. & Adachi, K. (2009) A parallel circuit of LIF signalling pathways maintains pluripotency of mouse ES cells. Nature, 460, 7251, (Jul 2009) 118-22, ISSN: 0028-0836

Oh, S.K.; Kim, H.S.; Park, Y.B.; Seol, H.W.; Kim, Y.Y.; Cho, M.S.; Ku, S.Y.; Choi, Y.M.; Kim, D.W. & Moon, S.Y. (2005) Methods for expansion of human embryonic stem cells. Stem Cells, 23, 5, (May 2005) 605-9, ISSN: 1066-5099

Okabe, S.; Forsberg-Nilsson, K.; Spiro, A.C.; Segal, M. & McKay, R.D. (1996) Development of neuronal precursor cells and functional postmitotic neurons from embryonic stem cells in vitro. Mech Dev, 59, 1, (Sep 1996) 89-102, ISSN: 0925-4773

Okano, H. (2002) Neural stem cells: progression of basic research and perspective for clinical application. Keio J Med, 51, 3, (Sep 2002) 115-28, ISSN: 1880-1293

Pain, B.; Clark, M.E.; Shen, M.; Nakazawa, H.; Sakurai, M.; Samarut, J. & Etches, R.J. (1996) Long-term in vitro culture and characterisation of avian embryonic stem cells with
multiple morphogenetic potentialities. Development, 122, 8, (Aug 1996) 2339-48, ISSN: 0950-1991

Parisi, S.; D’Andrea, D.; Lago, C.T.; Adamson, E.D.; Persico, M.G. & Minchiotti, G. (2003) Nodal-dependent Cripto signaling promotes cardiomyogenesis and redirects the neural fate of embryonic stem cells. J Cell Biol, 163, 2, (Oct 2003) 303-14, ISSN: 0021-9525

Pera, M.F.; Reubinoff, B. & Trounson, A. (2000) Human embryonic stem cells. J Cell Sci, 113 (Pt 1), (Jan 2000) 5-10, ISSN: 0021-9533

Potten, C.S. (1998) Stem cells in gastrointestinal epithelium: numbers, characteristics and death. Philos Trans R Soc Lond B Biol Sci, 353, 1370, (Jun 1998) 821-30, ISSN: 0962-8436

Prowse, A.B.; McQuade, L.R.; Bryant, K.J.; Van Dyk, D.D.; Tuch, B.E. & Gray, P.P. (2005) A proteome analysis of conditioned media from human neonatal fibroblasts used in the maintenance of human embryonic stem cells. Proteomics, 5, 4, (Mar 2005) 978-89, ISSN: 1615-9853

Rambhatla, L.; Chiu, C.P.; Kundu, P.; Peng, Y. & Carpenter, M.K. (2003) Generation of hepatocyte-like cells from human embryonic stem cells. Cell Transplant, 12, 1, 2003) 1-11, ISSN: 0963-6897

Ramiya, V.K.; Maraist, M.; Arfors, K.E.; Schatz, D.A.; Peck, A.B. & Cornelius, J.G. (2000) Reversal of insulin-dependent diabetes using islets generated in vitro from pancreatic stem cells. Nat Med, 6, 3, (Mar 2000) 278-82, ISSN: 1078-8956

Rastan, S. & Robertson, E.J. (1985) X-chromosome deletions in embryo-derived (EK) cell lines associated with lack of X-chromosome inactivation. J Embryol Exp Morphol, 90, (Dec 1985) 379-88, ISSN: 0022-0752

Reubinoff, B.E.; Itsykson, P.; Turetsky, T.; Pera, M.F.; Reinhartz, E.; Itzik, A. & Ben-Hur, T. (2001) Neural progenitors from human embryonic stem cells. Nat Biotechnol, 19, 12, (Dec 2001) 1134-40, ISSN: 1087-0156

Reubinoff, B.E.; Pera, M.F.; Fong, C.Y.; Trounson, A. & Bongso, A. (2000) Embryonic stem cell lines from human blastocysts: somatic differentiation in vitro. Nat Biotechnol, 18, 4, (Apr 2000) 399-404, ISSN: 1087-0156

Rideout, W.M., 3rd; Hochedlinger, K.; Kyba, M.; Daley, G.Q. & Jaenisch, R. (2002) Correction of a genetic defect by nuclear transplantation and combined cell and gene therapy. Cell, 109, 1, (Apr 2002) 17-27, ISSN: 0092-8674

Robertson, E.J. (1987) Embryo derived stem cell lines. In: Teratocarcinomas and Embryonic Stem Cells: A Practical Approach Robertson, E.J., 71-112, IRL Press, ISBN: 978-185221005, Oxford.

Rolletschek, A.; Chang, H.; Guan, K.; Czyz, J.; Meyer, M. & Wobus, A.M. (2001) Differentiation of embryonic stem cell-derived dopaminergic neurons is enhanced by survival-promoting factors. Mech Dev, 105, 1-2, (Jul 2001) 93-104, ISSN: 0925-4773

Sasaki, H. & Hogan, B.L. (1993) Differential expression of multiple fork head related genes during gastrulation and axial pattern formation in the mouse embryo. Development, 118, 1, (May 1993) 47-59, ISSN: 0950-1991

Sato, N.; Meijer, L.; Skalsounis, L.; Greengard, P. & Brivanlou, A.H. (2004) Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. Nat Med, 10, 1, (Jan 2004) 55-63, ISSN: 1078-8956
Schoonjans, L.; Albright, G.M.; Li, J.L.; Collen, D. & Moreadith, R.W. (1996) Pluripotential rabbit embryonic stem (ES) cells are capable of forming overt coat color chimeras following injection into blastocysts. Mol Reprod Dev, 45, 4, (Dec 1996) 439-43, ISSN: 1040-452X

Schroeder, T.; Fraser, S.T.; Ogawa, M.; Nishikawa, S.; Oka, C.; Bornkamm, G.W.; Honjo, T. & Just, U. (2003) Recombination signal sequence-binding protein Jkappa alters mesodermal cell fate decisions by suppressing cardiomyogenesis. Proc Natl Acad Sci U S A, 100, 7, (Apr 2003) 4018-23, ISSN: 0027-8424

Schuldiner, M.; Yanuka, O.; Itskovitz-Eldor, J.; Melton, D.A. & Benvenisty, N. (2000) Effects of eight growth factors on the differentiation of cells derived from human embryonic stem cells. Proc Natl Acad Sci U S A, 97, 21, (Oct 2000) 11307-12, ISSN: 0027-8424

Seale, P.; Asakura, A. & Rudnicki, M.A. (2001) The potential of muscle stem cells. Dev Cell, 1, 3, (Sep 2001) 333-42, ISSN: 1534-5807

Sedivy, J.M. & Joyner, A.L. (1992) Gene Targeting. W.H. Freeman and company, ISBN: 0-7167-7013-X, New York

Segev, H.; Fishman, B.; Ziskind, A.; Shulman, M. & Itskovitz-Eldor, J. (2004) Differentiation of human embryonic stem cells into insulin-producing clusters. Stem Cells, 22, 3, (2004) 265-74, ISSN: 1066-5099

Sellem, C.H.; Frain, M.; Erdos, T. & Sala-Trepat, J.M. (1984) Differential expression of albumin and alpha-fetoprotein genes in fetal tissues of mouse and rat. Dev Biol, 102, 1, (Mar 1984) 51-60, ISSN: 0012-1606

Simon, H.H.; Bhatt, L.; Gherbassi, D.; Sgado, P. & Alberi, L. (2003) Midbrain dopaminergic neurons: determination of their developmental fate by transcription factors. Ann N Y Acad Sci, 991, (Jun 2003) 36-47, ISSN: 0077-8923

Smith, A.G. (2001) Embryo-derived stem cells: of mice and men. Annu Rev Cell Dev Biol, 17, 2001) 435-62, ISSN: 1081-0706

Smith, A.G.; Nichols, J.; Robertson, M. & Rathjen, P.D. (1992) Differentiation inhibiting activity (DIA/LIF) and mouse development. Dev Biol, 151, 2, (Jun 1992) 339-51, ISSN: 0012-1606

Sukoyan, M.A.; Golubitsa, A.N.; Zhelezova, A.I.; Shilov, A.G.; Vatolin, S.Y.; Maximovsky, L.P.; Andreeva, L.E.; McWhir, J.; Pack, S.D.; Bayborodin, S.I. & et al. (1992) Isolation and cultivation of blastocyst-derived stem cell lines from American mink (Mustela vison). Mol Reprod Dev, 33, 4, (Dec 1992) 418-31, ISSN: 1040-452X
Sun, L.; Bradford, C.S.; Ghosh, C.; Collodi, P. & Barnes, D.W. (1995) ES-like cell cultures derived from early zebrafish embryos. Mol Mar Biol Biotechnol, 4, 3, (Sep 1995) 193-9, ISSN: 1053-6426

Takahashi, K.; Tanabe, K.; Ohnuki, M.; Narita, M.; Ichisaka, T.; Tomoda, K. & Yamanaka, S. (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell, 131, 5, (Nov 2007) 861-72, ISSN: 0092-8674

Takahashi, K. & Yamanaka, S. (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell, 126, 4, (Aug 2006) 663-76, ISSN: 0092-8674

Takahashi, T.; Lord, B.; Schulze, P.C.; Fryer, R.M.; Sarang, S.S.; Gullans, S.R. & Lee, R.T. (2003) Ascorbic acid enhances differentiation of embryonic stem cells into cardiac myocytes. Circulation, 107, 14, (Apr 2003) 1912-6, ISSN: 0009-7322

Theise, N.D.; Saxena, R.; Portmann, B.C.; Thung, S.N.; Yee, H.; Chiriboga, L.; Kumar, A. & Crawford, J.M. (1999) The canals of Hering and hepatic stem cells in humans. Hepatology, 30, 6, (Dec 1999) 1425-33, ISSN: 0270-9139

Thomson, J.A.; Itskovitz-Eldor, J.; Shapiro, S.S.; Waknitz, M.A.; Swiergiel, J.J.; Marshall, V.S. & Jones, J.M. (1998) Embryonic stem cell lines derived from human blastocysts. Science, 282, 5391, (Nov 1998) 1145-7, ISSN: 0036-8075

Tropepe, V.; Coles, B.L.; Chiasson, B.J.; Horsford, D.J.; Elia, A.J.; McInnes, R.R. & van der Kooy, D. (2000) Retinal stem cells in the adult mammalian eye. Science, 287, 5460, (Mar 2000) 2032-6, ISSN: 0036-8075

Tropepe, V.; Hitoshi, S.; Sirard, C.; Mak, T.W.; Rossant, J. & van der Kooy, D. (2001) Direct neural fate specification from embryonic stem cells: a primitive mammalian neural stem cell stage acquired through a default mechanism. Neuron, 30, 1, (Apr 2001) 65-78, ISSN: 0896-6273

Wakayama, S.; Hikichi, T.; Suetsugu, R.; Sakaide, Y.; Bui, H.T.; Mizutani, E. & Wakayama, T. (2007) Efficient establishment of mouse embryonic stem cell lines from single blastomeres and polar bodies. Stem Cells, 25, 4, (Apr 2007) 986-93, ISSN: 1066-5099

Wang, G.; Zhang, H.; Zhao, Y.; Li, J.; Cai, J.; Wang, P.; Meng, S.; Feng, J.; Miao, C.; Ding, M.; Li, D. & Deng, H. (2005) Noggin and bFGF cooperate to maintain the pluripotency of human embryonic stem cell lines in the absence of feeder layers. Biochem Biophys Res Commun, 330, 3, (May 2005) 394-42, ISSN: 0006-291X

Watt, F.M. (2001) Stem cell fate and patterning in mammalian epidermis. Curr Opin Genet Dev, 11, 4, (Aug 2001) 410-7, ISSN: 0959-437X

Watt, F.M. & Driskell, R.R. (2010) The therapeutic potential of stem cells. Philos Trans R Soc Lond B Biol Sci, 365, 1537, (Jan 2010) 155-63, ISSN: 0962-8436

Wells, D.N.; Misica, P.M.; Day, T.A. & Tervit, H.R. (1997) Production of cloned lambs from an established embryonic cell line: a comparison between in vivo- and in vitro-matured cytoplasts. Biol Reprod, 57, 2, (Aug 1997) 385-93, ISSN: 0006-3363

Wernig, M.; Meissner, A.; Cassady, J.P. & Jaenisch, R. (2008) c-Myc is dispensable for direct reprogramming of mouse fibroblasts. Cell Stem Cell, 2, 1, (Jan 2008) 10-2, ISSN: 1934-5909

Wernig, M.; Meissner, A.; Foreman, R.; Brambrink, T.; Ku, M.; Hochedlinger, K.; Bernstein, B.E. & Jaenisch, R. (2007) In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. Nature, 448, 7151, (Jul 2007) 318-24, ISSN: 0028-0836
Wichterle, H.; Lieberam, I.; Porter, J.A. & Jessell, T.M. (2002) Directed differentiation of embryonic stem cells into motor neurons. *Cell*, 110, 3, (Aug 2002) 385-97, ISSN: 0092-8674

Willert, K. & Jones, K.A. (2006) Wnt signaling: is the party in the nucleus? *Genes Dev*, 20, 11, (Jun 2006) 1394-404, ISSN: 0890-9369

Wu, M.Y. & Hill, C.S. (2009) Tgf-beta superfamily signaling in embryonic development and homeostasis. *Dev Cell*, 16, 3, (Mar 2009) 329-43, ISSN: 1534-5807

Xu, C.; Inokuma, M.S.; Denham, J.; Golds, K.; Kundu, P.; Gold, J.D. & Carpenter, M.K. (2001) Feeder-free growth of undifferentiated human embryonic stem cells. *Nat Biotechnol*, 19, 10, (Oct 2001) 971-4, ISSN: 1087-0156

Xu, C.; Liguori, G.; Persico, M.G. & Adamson, E.D. (1999) Abrogation of the Cripto gene in mouse leads to failure of postgastrulation morphogenesis and lack of differentiation of cardiomyocytes. *Development*, 126, 3, (Feb 1999) 483-94, ISSN: 0950-1991

Xu, C.; Police, S.; Rao, N. & Carpenter, M.K. (2002) Characterization and enrichment of cardiomyocytes derived from human embryonic stem cells. *Circ Res*, 91, 6, (Sep 2002) 501-8, ISSN: 0009-7330

Xu, R.H.; Peck, R.M.; Li, D.S.; Feng, X.; Ludwig, T. & Thomson, J.A. (2005) Basic FGF and suppression of BMP signaling sustain undifferentiated proliferation of human ES cells. *Nat Methods*, 2, 3, (Mar 2005) 185-90, ISSN: 1548-7091

Ye, W.; Shimamura, K.; Rubenstein, J.L.; Hynes, M.A. & Rosenthal, A. (1998) FGF and Shh signals control dopaminergic and serotonergic cell fate in the anterior neural plate. *Cell*, 93, 5, (May 1998) 755-66, ISSN: 0092-8674

Ying, Q.L.; Stavridis, M.; Griffiths, D.; Li, M. & Smith, A. (2003) Conversion of embryonic stem cells into neuroectodermal precursors in adherent monoculture. *Nat Biotechnol*, 21, 2, (Feb 2003) 183-6, ISSN: 1087-0156

Yu, J.; Vodyanik, M.A.; Smuga-Otto, K.; Antosiewicz-Bourget, J.; Frane, J.L.; Tian, S.; Nie, J.; Jonsdottir, G.A.; Ruotti, V.; Steward, R.; Slukvin, II & Thomson, J.A. (2007) Induced pluripotent stem cell lines derived from human somatic cells. *Science*, 318, 5858, (Dec 2007) 1917-20, ISSN: 0036-8075

Zambidis, E.T.; Peault, B.; Park, T.S.; Bunz, F. & Civin, C.I. (2005) Hematopoietic differentiation of human embryonic stem cells progresses through sequential hematopoietic, primitive, and definitive stages resembling human yolk sac development. *Blood*, 106, 3, (Aug 1 2005) 860-70, ISSN: 0006-4971

Zhang, S.C.; Wernig, M.; Duncan, I.D.; Brustle, O. & Thomson, J.A. (2001) *In vitro* differentiation of transplantable neural precursors from human embryonic stem cells. *Nat Biotechnol*, 19, 12, (Dec 2001) 1129-33, ISSN: 1087-0156

Zhang, W.; Lee, W.H. & Triarhou, L.C. (1996) Grafted cerebellar cells in a mouse model of hereditary ataxia express IGF-I system genes and partially restore behavioral function. *Nat Med*, 2, 1, (Jan 1996) 65-71, ISSN: 1078-8956
Pluripotent stem cells have the potential to revolutionise medicine, providing treatment options for a wide range of diseases and conditions that currently lack therapies or cures. This book describes methodological advances in the culture and manipulation of embryonic stem cells that will serve to bring this promise to practice.

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