22

Genome Stability in Embryonic Stem Cells

Paola Rebuzzini[1], Maurizio Zuccotti[2*],
Carlo Alberto Redi[1,3] and Silvia Garagna[1,4,5*]
[1]*Laboratorio di Biologia dello Sviluppo, Dipartimento di Biologia Animale, Università degli Studi di Pavia, Via Ferrata 9, 27100 Pavia,*
[2]*Sezione di Istologia ed Embriologia, Dipartimento di Medicina Sperimentale, Università degli Studi di Parma, Via Volturno 39, 43100 Parma*
[3]*Fondazione I.R.C.C.S. Policlinico San Matteo, Piazzale Golgi, 19, 27100 Pavia*
[4]*Centro di Ricerca Interdipartimentale di Ingegneria Tissutale, Università degli Studi di Pavia, Via Ferrata 1, 27100 Pavia*
[5]*Centro di Eccellenza in Biologia Applicata, Università degli Studi di Pavia, Via Ferrata 9, 27100 Pavia*
Italy

1. Introduction

The first embryonic stem cell (ESC) lines have been isolated at the beginning of the 1980s from the inner cell mass of mouse blastocysts (stage 5.5-7.5 days post-fertilization) with direct culture or immununosurgery by two groups of researchers working independently (mouse ESCs, mESCs, Evans & Kaufman, 1981; Martin 1981). It took more than a decade to obtain ESC lines from blastocysts of the primate rhesus monkey (Thomson et al., 1995), the common marmoset (*Callithrix jacchus*) (Thomson et al., 1996), human (hESCs; Thomson et al., 1998), dog (Hayes et al., 2008) and rat (Li et al., 2008; Buehr et al., 2008).
ESCs are undifferentiated, pluripotent and self-renewable cells that can be maintained *in vitro* in the same undifferentiated status over extended periods of culture. They grow in colonies and possess a high nucleus/cytoplasm ratio (Figure 1a). ESCs are characterized by the expression of specific transcription factors (OCT-4, SOX2 and NANOG), and surface markers (TRA-1-60, TRA-1-81, SSEA-3 and SSEA-4 in hESCs, and also Ssea-1 in mESCs) (Figure 1b′ and c′), by high telomerase expression and alkaline phosphatase activity (Figure 1d). If injected into a blastocyst, they are able to participate to foetal development and to the formation of the germ cell line; also, following their injection into immunodeficient mice, they form teratomas with derivatives of all three germ layers. Under appropriate *in vitro* culture conditions in suspension, ESCs form three-dimensional cell aggregates called embryoid bodies (EBs; Figure 1e), that differentiate into the three germ layers (ectoderm, mesoderm and endoderm; Figure 1f, g and h). Following the addition of bone morphogenetic protein 4 (BMP4) to the culture medium, it has been demonstrated that both hESCs (Xu et al. 2002) and mESCs (Hayashi et al., 2010) can differentiate into the trophoblast.

* Corresponding authors

Because of their plasticity and potential to differentiate in all the cell types, ESCs represent an important tool for investigating early development for the study of genetic disease and as a cellular *in vitro* model for screening the effects of new drugs or xenobiotics; and in regenerative medicine and tissue replacement after injury or disease. At this regard, many disorders such as blood and immune-system related genetic diseases, cancer diabetes, Parkinson's disease and spinal cord injuries could be potentially treated using a pluripotent stem cell therapy, even if technical problems of graft-*versus*-host disease associated with allogenic stem cell transplantation (histocompatibility problems) are not negligible (Guyette et al., 2010; Marr et al., 2010; Arenas, 2010). In 2006, a new type of mouse pluripotent cells, with characteristics very similar to ESCs, has been developed by the group of Yamanaka (Takahashi & Yamanaka, 2006). These cells, called induced pluripotent stem cells (iPSCs), are the result of genome reprogramming by the ectopic expression of four transcription factors (Oct-4, Sox2, c-Myc and Klf4) of differentiated fibroblasts. iPSCs exhibit ESCs morphology and growth properties; they are pluripotent, undifferentiated and express ESCs markers. iPSCs have also been subsequently generated from human, rhesus monkey and rat adult primary fibroblasts (Takahashi et al., 2007; Liu et al., 2008; Li et al., 2009) and, more recently, from human adult blood cells (Loh et al., 2009) and rat bone marrow (Liao et al., 2009).

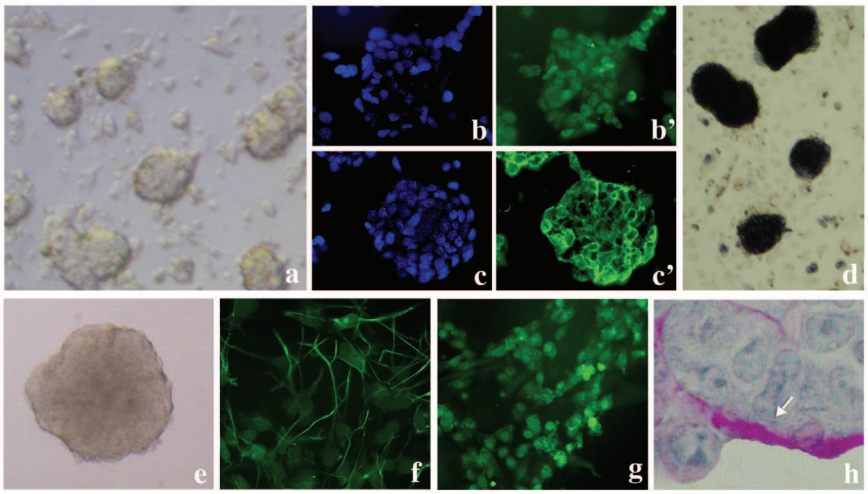


Fig. 1. Mouse embryonic stem cells and their derived embryoid bodies. Morfology of mESC colonies (a); immunocytochemical detection of Oct-4 (b') protein and Ssea-1 surface antigen (c') expression; alkaline phosphatase positive colonies (d); an embryoid body obtained after 5 days of mESCs differentiation (e); mESCs differentiated into cells of the ectoderm layer, expressing the Nestin marker (f); mESCs differentiated into cells of the mesoderm layer, expressing the Flk-1 marker (g); mESCs differentiated into cells of the endoderm layer, observed by histological examination of endodermal epithelial cells (arrow)

2. Loss of genome stability, the importance and consequences for ESCs

The mainteinance of the genomic stability is crucial for normal cell survival and cell growth. Genomic instability is a general term to describe the processes that can increase the rate of mutation of a population, enabling cells to develop new and aggressive phenotypes. Two are

the main mechanisms of instability: microsatellite and chromosomal instability (Lengauer et al., 1997). Microsatellite instability involves simple DNA base changes or tandemly repeated nucleotide sequences (microsatellite regions), whereas chromosomal instability involves whole chromosomes or large portions of them that are gained, lost or rearranged.

The maintenance of the correct chromosome complement is one of the most important necessity for ESCs, in particular for their possible therapeutical use. As other cell lines cultivated *in vitro*, ESCs are prone to accumulate karyotype abnormalities during long period of culture, although their mutation frequency is about 100 times lower when compared to differentiated cells, suggesting that ESCs have specialized mechanisms to preserve their genome integrity (Tichy & Stambrook, 2008).

3. Methods to study the chromosome complement

A chromosome aberration is either an incorrect number of chromosomes (that can occur as a consequence of an error during cell division) or a structural abnormality in one or more chromosomes. There are many types of chromosome anomalies, which can be organized into two groups: numerical or structural (Figure 2). An abnormal number of chromosomes is called aneuploidy and occurs when either one or more chromosomes are missing or gained. A structural abnormality is defined when the normal chromosome structure is altered (e.g., deletion, duplication, translocation etc.).

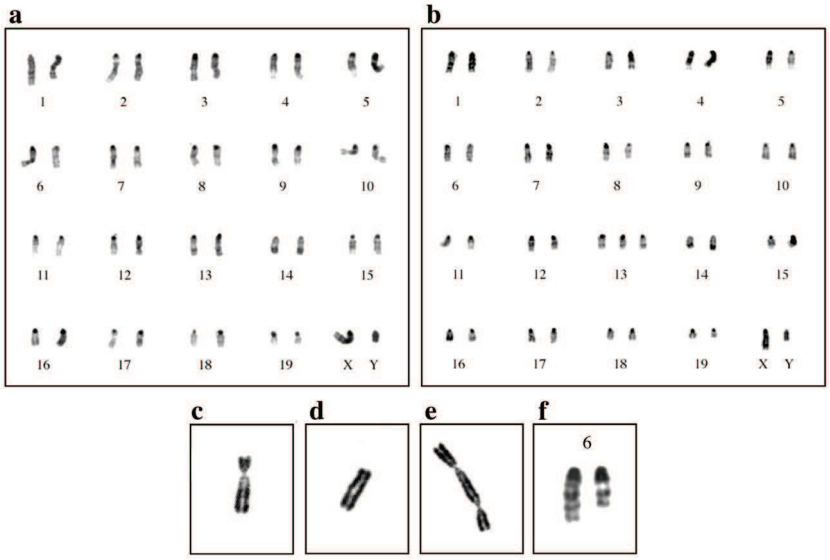


Fig. 2. Examples of numerical and structural chromosome abnormalities a mESC line. Reverted image of a DAPI-banded karyotype of a normal metaphase from a mESC line (a); numerical abnormal metaphase with a trisomy of chromosome 13 from a mESC line (b); structural chromosome abnormalities: metacentric chromosomes (c), chromosome fragment (d) dicentric chromosome (e), human chromosome 3 insertion (f), mouse chromosome 6 deletion (f)

Various types of methods are currently available to determine the chromosome complement and evaluate its integrity. Each technique has advantages and disadvantages in terms of

sensitivity, resolution and costs (Catalina et al., 2007). Classical simple banding techniques allow the regular check of the chromosome composition of the cell lines. For example, G-(Giemsa-stain) and DAPI-banding, providing 300-400 stained bands, permit both the identification of uncorrect chromosome numbers (aneuploidies) and structural chromosome abnormalities (e.g., translocations, deletions or insertions) of wide portions of chromosomes with a resolution of 5-10 Mb. Spectral karyotype (SKY technique) and multicolor fluorescent *in situ* hybridization (mFISH) represent an evolution of the conventional banding analysis (Schrock et al., 1996; Liyanage et al., 1996). Sky and mFISH allow the identification of each single chromosome with a higher resolution, approximately 1-2 Mb, when compared to classical cytogenetic methods and are usefull for the detection of submicroscopic deletions, insertions or DNA amplifications. However, to detect smaller genetic imbalances, the best techniques available at present are the array-based comparative genomic hybridization (array-CGH; Sanlaville et al., 2005) and the single nucleotide polymorphism array (SNP-array; Peiffer et al., 2006). The resolution of these techniques allows the detection of tiny aberrations (from 1 Mb to less than 100 kb) including homo- or hemizygous deletions, copy-neutral loss of heterozygosity, duplications and amplifications; however, these procedures are unable to evaluate the frequency within the cell population of a specific abnormality. Although these techniques shorten the whole screening procedure because they do not require cells blocked at metaphase, somehow, the costs of the equipment and consumables are an obstacle for their routinary use in the monitoring of chromosome stability.
In summary, the combination of both conventional and molecular cytogenetic technologies represents the best approach for the evaluation of the genomic integrity of a ESC lines.

4. Chromosome variation in human, primate and rodent ESCs

In the literature, a fine characterization of abnormalities and a potential explanation about their onset are present for both human and mouse ESCs. Some information is available also for iPSC cells, non-human primates and rat ESCs. A brief overview is reported below.
Human
hESCs can accumulate abnormalities when maintained in culture for few months. The chromosome changes observed affect more frequently chromosomes 12, 17, 20 and X. The reason why these chromosomes are more frequently involved is not clear, although it has been proposed that their alterations confer a selective and/or proliferative advantage to cells carring the mutations. The gain of part or of the entire chromosome 12 has been found in many hESC lines (i.e., BG01, BG02, BG03, H1, H7, H9, H14 and HS181) and observed in many indipendent laboratories (Brimble et al., 2001; Draper et al., 2004; Mitalipova et al., 2005; Ludwig et al., 2006; Imreh et al., 2006). The presence of an additional copy of chromosome 17 is another frequent abnormality found in hESCs (Brimble et al., 2001; Mitalipova et al., 2005). Sometimes associated with the gain of chromosome 12, the gain of the q arm of the chromosome 17 has been observed (Draper et al., 2004). Even if it is not really clear why these chromosomes are frequently involved in hESCs karyotypic changes, it has been suggested that the increased dosage of proteins coded by some genes located on chromosomes 12 and 17 could confer a selective advantage to cells carring these mutations. Human Stella-related (*STELLAR*), *NANOG*, the Growth differentiation factor-3 (*GDF3*) are stem cell pluripotency markers located on chromosome 12p (Clark et al., 2004) whose over-expression may participate to the maintainance of the pluripotent status (Spits et al., 2008). Similarly, the over-expression of *BIRC5* (that encodes for the anti-apoptotic survivin protein; Blum et al., 2009) or of hsa-mir-21 microRNA (involved in tumorigenesis, cancer

progression and a regulator of the anti-apoptotic *BCL2* gene; Caldas & Brenton, 2005), both located on chromosome 17, may confer a proliferation advantage.
The trisomy of chromosomes 12 and/or 17 is often associated with the X chromosome trisomy (Brimble et al., 2004; Inzunza et al., 2004; Mitalipova et al., 2005; Ludwig et al., 2006). Recently, Navarro and colleagues have demonstrated that in mESC the three pluripotency factors (Nanog, Oct4 and Sox2) bind and repress *Xist*, the master regulator of X inactivation, but it is not clear how the trisomy of this chromosome could confer a proliferative or selective advantage to cells (Navarro et al., 2008).
The gain of the entire or a part of chromosome 20 is an other typical chromosomal variation in hESCs (Rosler et al., 2004; Baker et al., 2007; Maitra et al., 2005; Spits et al., 2008; Lefort et al., 2008; Werbowetski-Ogilvie et al., 2009). It is known that the amplification of the region 20q11.2 is recurrent in many types of cancer (melanoma, Koynova et al., 2007; breast, Guan et al., 1996; lung, Tonon et al., 2005; bladder, Hurst et al., 2004) and the possible candidate genes that can increase cell proliferation, are *BCL2L1*, directly involved in cell death and proliferation, *DNMT3B*, important for the correct imprinting, and *POFUT1*, which is indispensable for *NOTCH* cascade signaling activation.
At present only a handful of papers has been published on the genomic integrity of human iPSCs. These pluripotent cells (derived from human adult fibroblasts; Takahashi et al., 2007; Lowry et al., 2008) usually own a normal karyotype during the early culture passages and they lack of hot spot instability regions. However, continuous passaging of iPSCs (e.g. derived from keratinocytes) resulted in the appearance of chromosomal abnormalities (46,XY,t(17;20)(p13;p11.2)) in 70% of the cells after 13 passages, involving the same chromosomes 17 and/or 20 frequently detected in hESCs (Aasen et al., 2008). Using human CGH Arrays, Chin and collaborators have observed few karyotypic alterations (the duplication of part of chromosome 8) in a late-passage (passage 44) in an iPSC line derived from a fibroblast line (Chin et al., 2009).

Non-human primates

Non-human primate ESC (nhpESC) lines are an important research tool for basic and applicative research. The rhesus macaque is physiologically and phylogenetically similar to human, and, therefore, it is a clinically relevant animal model for biomedical research. Even if a number of ESCs lines have been established from rhesus monkey (*Macaca mulatta*), common marmoset (*Callithrix jacchus*) and cynomolgus monkey (*Macaca fascicularis*) (Thomson et al., 1995; Thomsom et al., 1996; Nakatsuji and Suemori, 2002), very few studies have described their chromosome complement.
The little information available shows that using a serum-free medium and subculturing with trypsin, cynomolgus and rhesus monkey ESCs maintain a normal chromosome complement and pluripotency characterisitics even after over 1 year of continuous culture (Nakatsuji and Suemori, 2002). More recently, the cytogenetic analysis of 18 rhesus monkey ESC lines revealed that the majority (15) of them maintained a normal karyotype with a normal diploid chromosome number. The three unstable ESC lines (ORMES-1, -2, and -5) showed, even at low passages, structural abnormalities, such as translocations (t(11;16) and t(5;19) with der (18) t(1;18)), or invertions (inv (1)). It has been hypothesized that the collagenase-based dissociation technique, used for ORMES-1, -2, and -5, may have contributed to the onset of karyotypic abnormalities in these cell lines (Mitalipov et al., 2006).

Mouse

Unexpectedly, an accurate literature search showed that only a few papers described the genomic variation of mESCs during a long period of culture. In many mESC lines, no

recurrent chromosome abnormalities, but rather random alterations have been described by some laboratories (Longo et al., 1997; Guo et al., 2005; Sugawara et al. 2006; Rebuzzini et al., 2008a; Rebuzzini et al., 2008b).

The most complete analysis that has been made on mESCs was performed by Sugawara and colleagues (Sugawara et al., 2006). Following the observation of a total of 540 mESC lines, these authors showed that 66.5% of them presented a normal 2n=40 karyotype, whereas 15.9%, 9.1%, and 2.8% showed modal chromosomal numbers of 41, 42, and 39, respectively. Among 88 mESC lines, selected arbitrarily from the 540 lines, 60.2% showed a normal diploid karyotype, 51.4% showed a trisomy of chromosome 8, 14.3% had trisomy 8 in association with the loss of one sex chromosome, and 11.4% had trisomy 8 together with trisomy 11.

The chromosome complement of ESCs is important in contributing both to somatic cell chimaerism and to germ line transmission. Euploid mESCs cultured *in vitro* for up to 20 passages rapidly became severely aneuploid. Notably, when injected into the murine blastocyst, the percentage of euploid metaphases in the mESC clones correlates with the success obtained in the experiment: the more stable is the chromosome complement the higher is the number of chimeric embryos and pups obtained, and the higher is their the chimaerism. None of the mESC clones with more than 50% of chromosomally abnormal metaphases can be transmitted to the germline (Longo et al., 1997). Another confirmation that prolonged cell culture affects the normal diploid chromosomal composition of the population was reported by Guo and collaborators (Guo et al., 2005). Using mFISH analysis of four different mESC lines, they demonstrated that, although the morphology and the expression of stem markers appeared normal, two cell lines presented consistent numerical (41, 43, 44, sub- or tetraploid chromosome complement) and structural (trisomy of chromosomes 8, 12, 14 and 15, deletion of chromosome 6q and other aberrations with low frequency) aberrations (Guo et al., 2005). More recently, in our laboratory, we have analysed the chromosome complement of four indipendent mESC lines cultured for 3 months. In UPV04 mESC line about 60% of metaphases analysed were 2n=40 throughout the culture period. From passage 13, 50% of metaphases were euploid, with a correct chromosome complement and the remaining 50% showed gain or loss of entire chromosomes, both within the same passage and among different passages analysed. A very heterogeneous spectrum of abnormalites was described, indicating their continuous arising (Rebuzzini et al., 2008a). In other three mESC lines, named UPV02, UPV06 and UPV08, a progressive loss of euploid metaphases during culture has been observed and chromosome abnormalities, in particular metacentric chromosomes, accumulated at the latest passages analysed (passage 31, 29 and 22 for UPV02, UPV06 and UPV08, respectively). We observed that in coincidence with, or few passages after, the drop of euploidy, the alkaline phosphatase activity, one important ESC marker, was partially or totally lost (Rebuzzini et al., 2008b).

Rat

The rat ESCs (rESCs) are an important resource for the study of disease models, however, despite several temptatives (Brenin et al., 1997; Vassilieva et al., 2000) they have been derived only very recently (Buehr et al., 2008; Ueda et al., 2008; Zhao et al., 2010). In two cell lines derived by Buehr and collegues in 2008, a trisomy for chromosome 9 was described both by CGH and by FISH analysis. In two rESC lines, recently established from Wistar rat blastocysts, a normal number of chromosomes was observed at low passages (before passage 11, approximately 40% exhibit a normal karyotype), but a rapid accumulation of chromosomal abnormalities was described at later passages (up to 16 passages) (Ueda et al., 2008).

5. Possible causes of chromosome variations during culture

The variety of culture protocols applied in different laboratories working with ESCs may be the source of variations in cell differentiation and genome stability. Many papers published during the last decade described the presence of the feeder layer, the source of the serum (whether of animal or artificial origin) and the techniques used for cell passaging as the main and major factors affecting the maintainance of genome integrity during long culture periods. The majority of the data and information available on culture conditions are on human and mouse ESCs.

Generally, ESCs are derived and maintained *in vitro* with a co-culture protocol on a feeder layer of mitotically inactivated fibroblast cells (mouse embryonic or immortalized fibroblasts) or on defined supportive matrixes (i.e., gelatin, fibronectin or matrigel™). Whether using the former or the latter, genetic alterations were observed both in mESC and hESC lines (Cowan et al., 2004; Draper et al., 2004; Rosler et al., 2004; Mitalipova et al., 2005; Maitra et al., 2005; Guo et al., 2005; Longo et al., 2005; Imreh et al., 2006; Sugawara et al., 2006; Rebuzzini et al., 2008a; Rebuzzini et al., 2008b), suggesting that the presence or absence of a supporting cellular feeder layer can not exclude the onset of aberrations in the ESCs genome.

A fundamental component of the ESC medium is the serum of animal (calf or bovine) or artificial (knockout serum replacement of defined composition) origin. Despite the type of serum used, the genomic stability seems compromised. In a recent publication (Herszfeld et al., 2006) better results were obtained in the production of more stable hESCs when a serum replacement was used, likely because the use of artificial serum avoids the uncertainty of its composition which is frequently observed with animal-derived sera.

The technique used to detach ESCs for passaging seems to play a major role in the maintenance of their genomic stability. ESC colonies can be dissociated mechanically (i.e., pipetting in and out and flushing the medium until the colonies are detached and disgregated), enzymatically or by manual (i.e., colonies are cut and removed using a blade) dissection. The manual and mechanical dissection are preferentially used during hESCs subculturing, as, being less aggressive, they better preserve the genome integrity (Buzzard et al., 2004; Mitalipova et al., 2005). The manual dissection can introduce a bias due to the choice of the colonies and the skill of the researcher (Lefort et al., 2000). A modified enzymatic dissociation solution, consisting of 0.25% trypsin, 0.1% collagenase IV, 20% KSR, and 1 mM $CaCl_2$ in PBS, in combination with manual dissection for bulk passaging of hESCs has been proposed by Suemori and colleagues in 2006, demonstrating the maintenance of a normal chromosome complement after more than 100 passages in culture (Suemori et al., 2006).

6. Conclusions

Because of their characteristics, ESCs represent an important and unique biological resource for cell therapy and regenerative medicine, but also they are more and more envisioned as opening new routes for pharmacological research (Laustriat et al., 2010). As addressed in this review, the mainteinance of a correct chromosome complement is fundamental for the employment of these cells and a constant monitoring of their stability is required. We have produced an up-to-date summary of the litterature available on chromosome complement in ESCs of several different species, highlighting the need for world-wide guidelines that would restrict a rather fragmented and puzzled scenario. Given the actual culture

conditions used, the preservation of ESCs with a stable karyotype appears to be difficult. Clearly, a single culture protocol for all the species under study does not appear feasible; instead, each model animal will necessitate its own specific guidelines. Based on our own experience mutuated with that gathered from the litterature described above, following is a summary of some important start points that we believe should be taken on board when aiming to obtain an ESC line with low chromosomal variations: 1) use of a serum with a chemically defined composition; 2) manual dissection of ESC colonies; 3) routine monitoring of the chromosome complement throughout the culture period.

7. Acknowledgments

This work was supported by: Fondazione Banca del Monte di Lombardia contributo per la ricerca 2009, Fondazione I.R.C.C.S. Policlinico San Matteo di Pavia, Regione Lombardia, Alma Mater Ticinensis, Giovani Ricercatori GR 07-VB.

8. References

Aasen, T.; Raya, A.; Barrero, M.J.; Garreta, E.; Consiglio, A.; Gonzalez, F.; Vassena, R.; Bilić, J.; Pekarik, V.; Tiscornia, G.; Edel, M.; Boué, S.; Izpisúa Belmonte, J.C. (2008) Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. *Nat Biotechnol* Vol. 26, No. 11: 1276-1284.

Arenas, E. (2010) Towards stem cell replacement therapies for Parkinson's disease. *Biochem Biophys Res Commun* Vol. 396, No. 1: 152-6.

Baker, D.E.; Harrison, N.J.; Maltby, E.; Smith, K.; Moore, H.D.; Shaw, P.J.; Heath, P.R.; Holden, H.; Andrews, P.W. (2007) Adaptation to culture of human embryonic stem cells and oncogenesis in vivo. *Nat Biotechnol* Vol. 25, No. 2: 207-15.

Blum, B.; Bar-Nur, O.; Golan-Lev, T.; Benvenisty, N. (2009) The anti-apoptotic gene survivin contributes to teratoma formation by human embryonic stem cells. *Nat Biotechnol* Vol. 27, No. 3: 281-7.

Brenin, D.; Look, J.; Bader, M.; Hübner, N.; Levan, G.; Iannaccone, P. (1997) Rat embryonic stem cells: a progress report. *Transplant Proc* Vol., 29, No. 3: 1761-5.

Brimble, S.N.; Zeng, X.; Weiler, D.A.; Luo, Y.; Liu, Y.; Lyons, I.G.; Freed, W.J.; Robins, A.J.; Rao, M.S.; Schulz, T.C. (2004) Karyotypic stability, genotyping, differentiation, feeder-free maintenance, and gene expression sampling in three human embryonic stem cell lines derived prior to August 9, 2001. *Stem Cells Dev* Vol. 13, No. 6: 585-97.

Buehr, M.; Meek, S.; Blair, K.; Yang, J.; Ure, J.; Silva, J.; McLay, R.; Hall, J.; Ying, Q.L.; Smith, A. (2008) Capture of authentic embryonic stem cells from rat blastocysts. *Cell* Vol. 135, No. 7: 1287-98.

Buzzard, J.J.; Gough, N.M.; Crook, J.M.; Colman, A. (2004) Karyotype of human ES cells during extended culture. *Nat Biotechnol* Vol. 22, No. 4: 381-2.

Caldas, C.; Brenton, J.D. (2005) Sizing up miRNAs as cancer genes. *Nat Med* Vol. 11, No. 7: 712-4.

Catalina, P.; Cobo, F.; Cortés J.L.; Nieto, A.I.; Cabrera, C.; Montes, R.; Concha, A.; Menendez, P. (2007) Conventional and molecular cytogenetic diagnostic methods in stem cell research: a concise review. *Cell Biol Int* Vol. 31, No. 9: 861-9.

Chin, M.H.; Mason, M.J.; Xie, W.; Volinia, S.; Singer, M.; Peterson, C.; Ambartsumyan, G.; Aimiuwu, O.; Richter, L.; Zhang, J.; Khvorostov, I.; Ott, V.; Grunstein, M.; Lavon, N.; Benvenisty, N.; Croce, C.M.; Clark, A.T.; Baxter, T.; Pyle, A.D.; Teitell, M.A.;

Pelegrini, M.; Plath, K.; Lowry, W.E. (2009) Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. *Cell Stem Cell* Vol. 5, No. 1: 111-23.

Clark, A.T.; Rodriguez, R.T.; Bodnar, M.S.; Abeyta, M.J.; Cedars, M.I.; Turek, P.J.; Firpo, M.T.; Reijo Pera, R.A. (2004) Human STELLAR, NANOG, and GDF3 genes are expressed in pluripotent cells and map to chromosome 12p13, a hotspot for teratocarcinoma. *Stem Cells* Vol. 22, No. 2: 169-79.

Cowan, C.A.; Klimanskaya, I.; McMahon, J.; Atienza, J.; Witmyer, J.; Zucker, J.P.; Wang, S.; Morton, C.C.; McMahon, A.P.; Powers, D.; Melton, D.A. (2004) Derivation of embryonic stem-cell lines from human blastocysts. *N Engl J Med* Vol. 350, No. 13: 1353-6.

Draper, J.S.; Moore, H.D.; Ruban, L.N.; Gokhale, P.J.; Andrews, P.W. (2004) Culture and characterization of human embryonic stem cells. *Stem Cells Dev* Vol. 13, No. 4: 325-36.

Draper, J.S.; Smith, K.; Gokhale, P.; Moore, H.D.; Maltby, E.; Johnson, J.; Meisner, L.; Zwaka, T.P.; Thomson, J.A.; Andrews, P.W. (2004) Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells. *Nat Biotechnol* Vol. 22, No. 1: 53-4.

Evans, M.J.; Kaufman, M.H. (1981) Establishment in culture of pluripotential cells from mouse embryos. *Nature* Vol. 292, No. 5819: 154-6.

Guan, X.Y.; Xu. J.; Anzick, S.L.; Zhang, H.; Trent, J.M.; Meltzer, P.S. (1996) Hybrid selection of transcribed sequences from microdissected DNA: isolation of genes within amplified region at 20q11-q13.2 in breast cancer. *Cancer Res* Vol. 56, No. 15: 3446-50.

Guo, J.; Jauch, A.; Heidi, H.G.; Schoell, B.; Erz, D.; Schrank, M.; Janssen, J.W. (2005) Multicolor karyotype analyses of mouse embryonic stem cells. *In Vitro Cell Dev Biol Anim* Vol. 41, No. 8-9: 278-83.

Guyette, J.P.; Cohen, I.S.; Gaudette, G.R. (2010) Strategies for regeneration of heart muscle. *Crit Rev Eukaryot Gene Expr* Vol. 20, No. 1: 35-50.

Hayashi, Y.; Furue, M.K.; Tanaka, S.; Hirose, M.; Wakisaka, N.; Danno, H.; Ohnuma, K.; Oeda, S.; Aihara, Y.; Shiota, K.; Ogura, A.; Ishiura, S.; Asashima, M. (2010) BMP4 induction of trophoblast from mouse embryonic stem cells in defined culture conditions on laminin. *In Vitro Cell Dev Biol Anim* Vol. 46, No. 5: 416-30.

Hayes, B.; Fagerlie, S.R.; Ramakrishnan, A.; Baran, S.; Harkey, M.; Graf, L.; Bar, M.; Bendoraite, A.; Tewari, M.; Torok-Storb, B. (2008) Derivation, characterization, and *in vitro* differentiation of canine embryonic stem cells. *Stem Cells* Vol. 26, No. 2: 465-73.

Herszfeld, D.; Wolvetang, E.; Langton-Bunker, E.; Chung, T.L.; Filipczyk, A.A.; Houssami, S.; Jamshidi, P.; Koh, K.; Laslett, A.L.; Michalska, A.; Nguyen, L.; Reubinoff, B.E.; Tellis, I.; Auerbach, J.M.; Ording, C.J.; Looijenga, L.H.; Pera, M.F. (2006) CD30 is a survival factor and a biomarker for transformed human pluripotent stem cells. *Nat Biotechnol* Vol. 24, No. 3: 351-7.

Hurst, C.D.; Fiegler, H.; Carr, P.; Williams, S.; Carter, N.P.; Knowles, M.A. (2004) High-resolution analysis of genomic copy number alterations in bladder cancer by microarray-based comparative genomic hybridization. *Oncogene* Vol. 23, No. 12: 2250-63.

Imreh, M.P.; Gertow, K.; Cedervall, J.; Unger, C.; Holmberg, K.; Szöke, K.; Csöregh, L.; Fried, G.; Dilber, S.; Blennow, E.; Ahrlund-Richter, L. (2006) In vitro culture conditions favoring selection of chromosomal abnormalities in human ES cells. *J Cell Biochem* Vol. 99, No. 2: 508-16.

Inzunza, J.; Sahlén, S.; Holmberg, K.; Strömberg, A.M.; Teerijoki, H.; Blennow, E.; Hovatta, O.; Malmgren, H. (2004) Comparative genomic hybridization and karyotyping of human embryonic stem cells reveals the occurrence of an isodicentric X chromosome after long-term cultivation. *Mol Hum Reprod* Vol.10, No. 6: 461-6.

Koynova, D.K.; Jordanova, E.S.; Milev, A.D.; Dijkman, R.; Kirov, K.S.; Toncheva, D.I.; Gruis, N.A. (2007) Gene-specific fluorescence *in-situ* hybridization analysis on tissue microarray to refine the region of chromosome 20q amplification in melanoma. *Melanoma Res* Vol. 17, No. 1: 37-41.

Laustriat, D.; Gide, J.; Peschanski, M. (2010) Human pluripotent stem cells in drug discovery and predictive toxicology. *Biochem Soc Trans* Vol. 38, No. 4: 1051-7.

Lengauer, C.; Kinzler, K.W.; Vogelstein, B. (1997) Genetic instability in colorectal cancers. *Nature* Vol. 386, No. 6625: 623-7.

Lefort, N.; Feyeux, M.; Bas, C.; Féraud, O.; Bennaceur-Griscelli, A.; Tachdjian, G.; Peschanski, M.; Perrier, A.L. (2008) Human embryonic stem cells reveal recurrent genomic instability at 20q11.21. *Nat Biotechnol* Vol. 26, No. 12: 1364-6.

Lefort, N.; Perrier, A.L.; Laâbi, Y.; Varela, C.; Peschanski, M. (2009) Human embryonic stem cells and genomic instability. *Regen Med* Vol. 4, No. 6: 899-909.

Li, P.; Tong, C.; Mehrian-Shai, R.; Jia, L.; Wu, N.; Yan, Y.; Maxson, R.E.; Schulze, E.N.; Song, H.; Hsieh, C.L.; Pera, M.F.; Ying, Q.L. (2008) Germline competent embryonic stem cells derived from rat blastocysts. *Cell* Vol. 135, No. 7: 1299-310.

Li, W.; Wei, W.; Zhu, S.; Zhu, J.; Shi, Y.; Lin, T.; Hao, E.; Hayek, A.; Deng, H.; Ding, S. (2009) Generation of rat and human induced pluripotent stem cells by combining genetic reprogramming and chemical inhibitors. *Cell Stem Cell* Vol. 4, No. 1: 16-9.

Liao, J.; Cui, C.; Chen, S.; Ren, J.; Chen, J.; Gao, Y.; Li, H.; Jia, N.; Cheng, L.; Xiao, H.; Xiao, L. (2009) Generation of induced pluripotent stem cell lines from adult rat cells. *Cell Stem Cell* Vol. 4, No. 1: 11-5.

Liu, H.; Zhu, F.; Yong, J.; Zhang, P.; Hou, P.; Li, H.; Jiang, W.; Cai, J.; Liu, M.; Cui, K.; Qu, X.; Xiang, T.; Lu, D.; Chi, X.; Gao, G.; Ji, W.; Ding, M.; Deng, H. (2008) Generation of induced pluripotent stem cells from adult rhesus monkey fibroblasts. *Cell Stem Cell* Vol. 3, No. 6: 587-90.

Liyanage, M.; Coleman, A.; du Manoir, S.; Veldman, T.; McCormack, S.; Dickson, R.B.; Barlow, C.; Wynshaw-Boris, A.; Janz, S.; Wienberg, J.; Ferguson-Smith, M.A.; Schröck, E.; Ried, T. (1996) Multicolour spectral karyotyping of mouse chromosomes. *Nat Genet* Vol. 14, No. 3: 312-5.

Loh, Y.H.; Agarwal, S.; Park, I.H.; Urbach, A.; Huo, H.; Heffner, G.C.; Kim, K.; Miller, J.D.; Ng, K.; Daley, G.Q. (2009) Generation of induced pluripotent stem cells from human blood. *Blood* Vol. 113, No. 22: 5476-9.

Longo, L.; Bygrave, A.; Grosveld, F.G.; Pandolfi PP. (1997) The chromosome make-up of mouse embryonic stem cells is predictive of somatic and germ cell chimaerism. *Transgenic Res* Vol. 6, No. 5: 321-8.

Lowry, W.E.; Richter, L.; Yachechko, R.; Pyle, A.D.; Tchieu, J.; Sridharan, R.; Clark, A.T.; Plath, K. (2008) Generation of human induced pluripotent stem cells from dermal fibroblasts. *Proc Natl Acad Sci U S A* Vol. 105, No. 8: 2883-8.

Ludwig, T.E.; Levenstein, M.E.; Jones, J.M.; Berggren, W.T.; Mitchen, E.R.; Frane, J.L.; Crandall, L.J.; Daigh, C.A.; Conard, K.R.; Piekarczyk, M.S.; Llanas, R.A.; Thomson, J.A. (2006) Derivation of human embryonic stem cells in defined conditions. *Nat Biotechnol* Vol. 24, No. 2: 185-7.

Maitra, A.; Arking, D.E.; Shivapurkar, N.; Ikeda, M.; Stastny, V.; Kassauei, K.; Sui, G.; Cutler, D.J.; Liu, Y.; Brimble, S.N.; Noaksson, K.; Hyllner, J.; Schulz, T.C.; Zeng, X.; Freed, W.J.; Crook, J.; Abraham, S.; Colman, A.; Sartipy, P.; Matsui, S.; Carpenter, M.; Gazdar, A.F.; Rao, M.; Chakravarti, A. (2005) Genomic alterations in cultured human embryonic stem cells. *Nat Genet* Vol. 37, No. 10: 1099-103.

Marr, R.A.; Thomas, R.M.; Peterson, D.A. (2010) Insights into neurogenesis and aging: potential therapy for degenerative disease? *Future Neurol* Vol. 5, No. 4: 527-541.

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* Vol. 78, No. 12: 7634-8.

Mitalipova, M.M.; Rao, R.R.; Hoyer, D.M.; Johnson, J.A.; Meisner, L.F.; Jones, K.L.; Dalton, S.; Stice, S.L. (2005) Preserving the genetic integrity of human embryonic stem cells. *Nat Biotechnol* Vol. 23, No. 1: 19-20.

Mitalipov, S.; Kuo, H.C.; Byrne, J.; Clepper, L.; Meisner, L.; Johnson, J.; Zeier, R.; Wolf, D. (2006) Isolation and characterization of novel rhesus monkey embryonic stem cell lines. *Stem Cells* Vol. 24; No. 10: 2177-86.

Nakatsuji, N.; Suemori, H. (2002) Embryonic stem cell lines of nonhuman primates. *ScientificWorldJournal* Vol. 26, No. 2: 1762-73.

Navarro, P.; Chambers, I.; Karwacki-Neisius, V.; Chureau, C.; Morey, C.; Rougeulle, C.; Avner, P. (2008) Molecular coupling of Xist regulation and pluripotency. *Science* Vol. 321, No. 5896: 1693-5.

Peiffer, D.A.; Le, J.M.; Steemers, F.J.; Chang, W.; Jenniges, T.; Garcia, F.; Haden, K.; Li, J.; Shaw, C.A.; Belmont, J.; Cheung, S.W.; Shen, R.M.; Barker, D.L.; Gunderson, K.L. (2006) High-resolution genomic profiling of chromosomal aberrations using Infinium whole-genome genotyping. *Genome Res* Vol. 16, No. 9: 1136-48.

Rebuzzini, P.; Neri, T.; Mazzini, G.; Zuccotti, M.; Redi, C.A.; Garagna, S. (2008a) Karyotype analysis of the euploid cell population of a mouse embryonic stem cell line revealed a high incidence of chromosome abnormalities that varied during culture. *Cytogenet Genome Res* Vol. 121, No. 1: 18-24.

Rebuzzini, P.; Neri, T.; Zuccotti, M.; Redi, C.A.; Garagna, S. (2008b) Chromosome number variation in three mouse embryonic stem cell lines during culture. *Cytotechnology* Vol. 58, No. 1: 17-23.

Rosler, E.S.; Fisk, G.J.; Ares, X.; Irving, J.; Miura. T.; Rao, M.S.; Carpenter, M.K. (2004) Long-term culture of human embryonic stem cells in feeder-free conditions. *Dev Dyn* Vol. 229, No. 2: 259-74.

Sanlaville, D.; Lapierre, J.M.; Turleau, C.; Coquin, A.; Borck, G.; Colleaux, L.; Vekemans, M.; Romana, S.P. (2005) Molecular karyotyping in human constitutional cytogenetics. *Eur J Med Genet* Vol. 48, No. 3: 214-31.

Schröck, E.; du Manoir, S.; Veldman, T.; Schoell, B.; Wienberg, J.; Ferguson-Smith, M.A.; Ning, Y.; Ledbetter, D.H.; Bar-Am, I.; Soenksen, D.; Garini, Y.; Ried, T. (1996) Multicolor spectral karyotyping of human chromosomes. *Science* Vol. 273, No. 5274: 494-7.

Spits, C.; Mateizel, I.; Geens, M.; Mertzanidou, A.; Staessen, C.; Vandeskelde, Y.; Van der Elst, J.; Liebaers, I.; Sermon, K. (2008) Recurrent chromosomal abnormalities in human embryonic stem cells. *Nat Biotechnol* Vol. 26, No. 12: 1361-3.

Suemori, H.; Yasuchika, K.; Hasegawa, K.; Fujioka, T.; Tsuneyoshi, N.; Nakatsuji, N. (2006) Efficient establishment of human embryonic stem cell lines and long-term

maintenance with stable karyotype by enzymatic bulk passage. *Biochem Biophys Res Commun* Vol. 345, No. 3: 926-32.

Sugawara, A.; Goto, K.; Sotomaru, Y.; Sofuni, T.; Ito, T. (2006) Current status of chromosomal abnormalities in mouse embryonic stem cell lines used in Japan. *Comp Med* Vol. 56, No. 1: 31-4.

Takahashi, K.; Yamanaka, S. (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* Vol. 126, No. 4: 663-76.

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* Vol. 131, No. 5: 861-72.

Thomson, J.A.; Kalishman, J.; Golos, T.G.; Durning, M.; Harris, C.P.; Becker, R.A.; Hearn, J.P. (1995) Isolation of a primate embryonic stem cell line. *Proc Natl Acad Sci U S A* Vol. 92, No. 17: 7844-8.

Thomson, J.A.; Kalishman, J.; Golos, T.G.; Durning, M.; Harris, C.P.; Hearn, J.P. (1996) Pluripotent cell lines derived from common marmoset (*Callithrix jacchus*) blastocysts. *Biol Reprod* Vol. 55, No. 2: 254-9.

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* Vol. 282, No. 5391: 1145-7.

Tonon, G.; Wong, K.K.; Maulik, G.; Brennan, C.; Feng, B.; Zhang, Y.; Khatry, D.B.; Protopopov, A.; You, M.J.; Aguirre, A.J.; Martin, E.S.; Yang, Z.; Ji, H.; Chin, L.; Depinho, R.A. (2005) High-resolution genomic profiles of human lung cancer. *Proc Natl Acad Sci U S A* Vol. 102, No. 27: 9625-30.

Ueda, S.; Kawamata, M.; Teratani, T.; Shimizu, T.; Tamai, Y.; Ogawa, H.; Hayashi, K.; Tsuda, H.; Ochiya, T. (2008) Establishment of rat embryonic stem cells and making of chimera rats. *PLoS One* Vol. 3, No. 7:e2800.

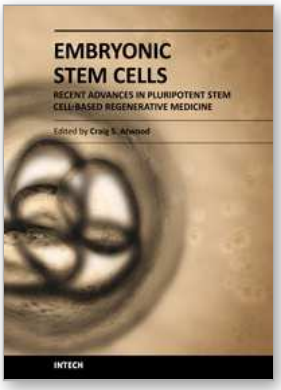
Vassilieva, S.; Guan, K.; Pich, U.; Wobus, A.M. (2000) Establishment of SSEA-1- and Oct-4-expressing rat embryonic stem-like cell lines and effects of cytokines of the IL-6 family on clonal growth. *Exp Cell Res* Vol. 258, No. 2: 361-73.

Werbowetski-Ogilvie, T.E.; Bossé, M.; Stewart, M.; Schnerch, A.; Ramos-Mejia, V.; Rouleau, A.; Wynder, T.; Smith, M.J.; Dingwall, S.; Carter, T.; Williams, C.; Harris, C.; Dolling, J.; Wynder, C.; Boreham, D.; Bhatia, M. (2009) Characterization of human embryonic stem cells with features of neoplastic progression. *Nat Biotechnol* Vol. 27, No. 1: 91-7.

Xu R, H.; Chen, X.; Li D. S.; Li, R.; Addicks, G. C.; Glennon, C.; Zwaka, T. P.; Thomson J.A. (2002) BMP4 initiates human embryonic stem cell differentiation to trophoblast. *Nat Biotechnol Vol*. 20, No. 12: 1261– 126.

Ying, Q.L.; Nichols, J.; Chambers, I.; Smith, A. (2003) BMP induction of Id proteins suppresses differentiation and sustains embryonic stem cell self-renewal in collaboration with STAT3. *Cell* Vol. 115, No. 3: 281-92.

Zhao, X.; Lv, Z.; Liu, L.; Wang, L.; Tong, M.; Zhou, Q. (2010) Derivation of embryonic stem cells from Brown Norway rats blastocysts. *J Genet Genomics* Vol. 37, No. 7: 467-73.



Embryonic Stem Cells - Recent Advances in Pluripotent Stem Cell-Based Regenerative Medicine

Edited by Prof. Craig Atwood

ISBN 978-953-307-198-5
Hard cover, 410 pages
Publisher InTech
Published online 26, April, 2011
Published in print edition April, 2011

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 recent advances in the generation of tissue specific cell types for regenerative applications, as well as the obstacles that need to be overcome in order to recognize the potential of these cells.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Paola Rebuzzini, Maurizio Zuccotti, Carlo Alberto Redi and Silvia Garagna (2011). Genome Stability in Embryonic Stem Cells, Embryonic Stem Cells - Recent Advances in Pluripotent Stem Cell-Based Regenerative Medicine, Prof. Craig Atwood (Ed.), ISBN: 978-953-307-198-5, InTech, Available from: http://www.intechopen.com/books/embryonic-stem-cells-recent-advances-in-pluripotent-stem-cell-based-regenerative-medicine/genome-stability-in-embryonic-stem-cells

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821