We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter 14

Epigenetic Instability in Embryonic Stem Cells

Takuro Horii and Izuho Hatada

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/54367

1. Introduction

Embryonic stem (ES) cells constitute a very important tool for regenerative medicine today. Human ES cells, in particular, are almost all derived from embryos obtained by *in vitro* fertilization (IVF) followed by *in vitro* culture (IVC); however, such *in vitro* manipulated embryos often show epigenetic abnormalities in imprinted genes that can lead to the development of various diseases. We recently reported that epigenetic differences occurred between ES cells derived from *in vivo* developed embryos (Vivo ES) and ES cells derived from *in vitro* manipulated embryos (Vitro ES) [1]. In addition, we found that the DNA methylation state of uniparental and somatic cell nuclear transfer (SCNT) ES cells exhibits epigenetic instability during *in vitro* culture [2]. In this chapter, we review studies that have examined the epigenetic instability of ES cells during generation and maintenance cultures, and discuss the candidate factors that may be responsible for this epigenetic instability.

2. Epigenetic regulation by DNA methylation

In vertebrate genomic DNA, the 5' cytosine residues in CpG sequences are often methylated [3]. DNA methylation plays an essential role in the normal development of mammalian embryos by regulating gene expression through genomic imprinting and X chromosome inactivation, and confers genomic stability [4-7]. In this chapter, we focus primarily on genomic imprinting, which is the preferential silencing of one of the parental alleles of a gene by epigenetic DNA methylation since epigenetic modifications to some imprinted genes cause diseases such as Beckwith-Wiedemann syndrome and Prader-Willie syndrome. For example, the expression level of the *H19* imprinted gene is regulated by an upstream differentially methylated region (DMR), and epigenetic alterations to the DMR result in Beckwith-Wiedemann syndrome [8-10]. The *H19* mRNA is transcribed from the unmethylated maternal al-

lele but is not transcribed from the methylated paternal allele (Fig. 1). In contrast, DMRs of *Peg1* (Mest), *Snrpn* and *Igf2r* are methylated in the maternal allele and unmethylated in the paternal allele. Genomic imprinting is very stable except for the period when the reprogramming of genomic imprinting takes place in germline cells [11]. For the establishment and maintenance of DNA methylation, the cytosine-guanine (CpG) DNA methyltransferases (Dnmts), Dnmt1, Dnmt3a, and Dnmt3b, are the main factors that coordinately regulate CpG methylation in the genome [12-14]. Dnmt1 is involved in maintenance activity, while Dnmt3a and Dnmt3b are responsible primarily for the creation of new methylation patterns.

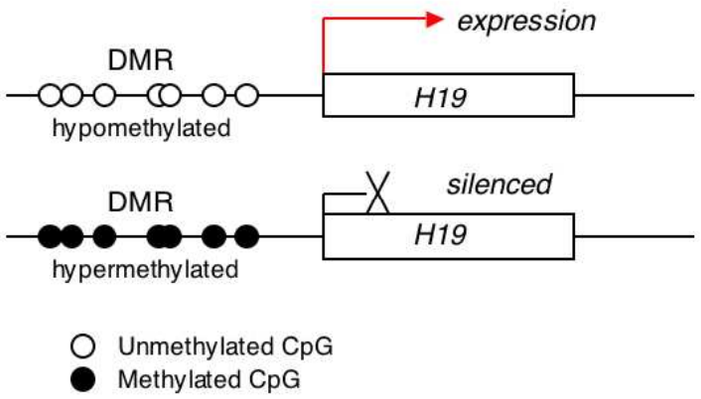

Figure 1. Regulation of gene expression in the *H19* imprinted gene.

3. Epigenetic instability in preimplantation embryos

In general, ES cells, especially human ES cells, are generated from blastocyst stage embryos that are produced by *in vitro* manipulations such as IVF and IVC. However, *in vitro* manipulated embryos may already possess epigenetic abnormalities because the culture conditions of fertilized embryos can influence the methylation state. For example, a sub-optimal culture medium (e.g., Whitten's medium) can cause aberrant genomic imprinting of the *H19* gene [15], and culture medium supplemented with fetal calf serum alters mRNA expression of imprinted genes [16]. Our recent study suggests that altered DNA methylation due to IVC conditions occurs not only in imprinted genes but also in genome-wide repetitive sequences, such as major and minor satellite sequences [17]. Thus, alteration of DNA methylation can occur in response to various factors, from the moment when embryos are collected from the oviducts or uterus.

4. Epigenetic instability in ES cells during prolonged culture

ES cells are established from the inner cell mass (ICM) of blastocyst stage embryos [18,19]. Once ES cell lines are established, they can be maintained for long periods of time and used for sever-

http://dx.doi.org/10.5772/54367

al applications. However, ES cells lose their pluripotency during prolonged *in vitro* culture [20]. Several studies indicate that the accumulation of epigenetic alterations over time is correlated with the loss of pluripotency in ES cells. Dean *et al*. reported that epigenetic alterations that occur in ES cells persist to later developmental stages and are associated with aberrant phenotypes in completely ES cell-derived mice [21]. Humpherys *et al*. show that variation in imprinted gene expression is observed in most cloned mice derived from ES cell donors, even those derived from ES cells of the same subclone [22]. Such epigenetic drift of imprinted genes was also observed in our experiments during prolonged culture of mouse ES cells (Fig. 2): DNA methylation of four imprinted genes, *Peg1*, *Snrpn*, *Igf2r* and *H19*, was unstable during cell culture (P3-30), even in the same cell line, over time. Minoguchi and Iba reported that retroviral DNA that is introduced into mouse ES cells is progressively silenced by DNA methylation; however, a substantial amount of retroviral DNA is reversibly reactivated by DNA demethylation [23]. Such epigenetic drift has also been observed in human ES cells, depending on the method of establishment and the culture conditions [24].

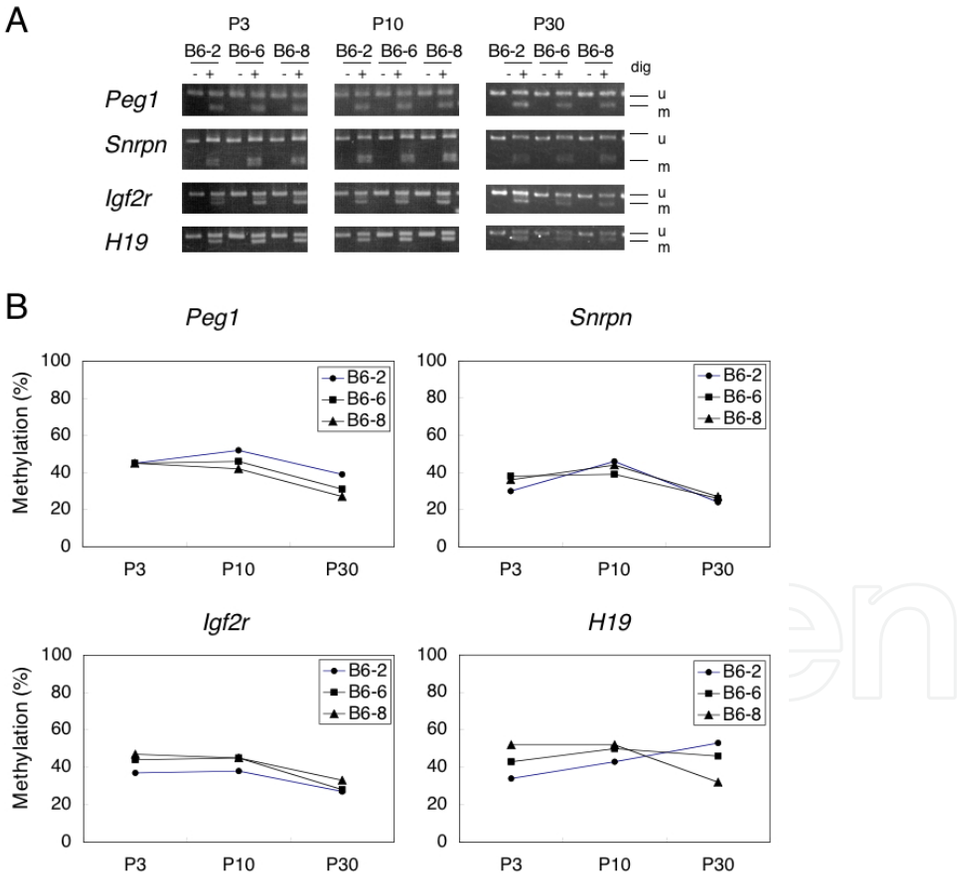

Figure 2. Epigenetic drift of imprinting methylations in fertilized embryo-derived ES cells. A. Combined bisulfite restriction analysis (COBRA) was conducted for three fertilized embryo-derived ES cell lines (B6-2, B6-6 and B6-8) during prolonged *in vitro* culture (P3, P10 and P30). The maternally methylated imprinted genes *Peg1*, *Snrpn* and *Igf2r*, and the paternally methylated imprinted gene, *H19*, were examined. B. Summary of imprinting methylations during prolonged culture of ES cells. dig, digestion by restriction enzymes; u, unmethylated PCR products; m, methylated PCR products.

5. Epigenetic differences between male and female ES cells

Large differences in epigenetic drift have been observed between male (XY) and female (XX) mouse ES cells. Global demethylation, including imprinted genes and satellite repeats, occurred more frequently in female ES cell lines compared to male ES cell lines [21, 25]. This global demethylation reflects the number and state of X chromosomes in ES cells. In general, both X chromosomes are active in female ES cells, whereas male ES cells have only one active X chromosome. The X chromosome state in female ES cells is thought to lead to downregulation of DNA methyltransferases (Dnmt3a and Dnmt3b) and, ultimately, to global hypomethylation [25]. Thus, DNA methylation of imprinted genes and repetitive sequences are gained or lost at high rates even in clonal populations of ES cells, and these alterations may have deleterious effects on phenotypes of ES cell-derived animals or tissues.

6. Epigenetic differences between *vivo* and *vitro* ES cells

6.1. Methylation state of *vivo* and *vitro* ES cells

In human ES cells, several studies have recently provided evidence for the efficient induction of endoderm, mesoderm, and ectoderm, and many of their downstream derivatives [26], and these reports offer broad possibilities for regenerative medicine. However, all human ES cell lines are established from *in vitro* manipulated embryos that often show abnormal genomic imprinting, which can lead to an increase in the frequency of diseases. Therefore, we have compared the methylation state of imprinted genes and the gene expression patterns of both Vivo and Vitro ES cell lines in mice [1].

Although the genomic imprinting is maintained during preimplantation development, normal imprinting can occasionally be disrupted in preimplantation embryos during IVC, resulting in biallelic expression of the *H19* gene [15,27]. To investigate whether Vitro ES cells take on abnormal imprinting from IVC blastocysts, we performed methylation analysis of the *H19* DMR for early passage (P2) cells (Fig. 3). COBRA analysis shows that the *H19* DMR is significantly demethylated in Vitro ES cells compared to Vivo ES cells. The *Igf2r* DMR2 also showed significant differences among Vitro vs. Vivo ES cells, but significant differences in the methylation of *Snrpn* and the major satellite repeats were not detected.

In additional experiments, both Vivo and Vitro ES cells were passaged several more times, and the methylation state of imprinted genes and satellite repeats was investigated at later passages (P5) (Fig. 3). Results from COBRA analysis at P5 showed no significant differences between Vivo and Vitro ES cells. Even Vivo ES cells exhibited highly demethylated alleles. In contrast, some Vitro ES cells had an almost normally methylated allele. This result indicates that the methylation state of ES cells at later passages depends more on the character of the individual cell lines than on the origin of the ES cells.

http://dx.doi.org/10.5772/54367

6.2. Gene expression of *vivo* and *vitro* ES cells

We assessed gene expression patterns in ES cells at early and late passages by quantitative real-time RT-PCR. The expression of *Oct3/4* mRNA, a pluripotent cell marker, was significantly higher in early passage Vivo ES cells than in Vitro ES cells, whereas other pluripotent marker genes, *Nanog* and *Stella*, showed no significant differences in expression levels between the two types of ES cells. Among the methylation-related genes, mRNA expression of the *de novo* DNA methyltransferase, Dnmt3b, was significantly higher in Vivo ES cells. Expression of growth arrest and DNA damage-inducible protein 45 beta (Gadd45*b*), which is a putative demethylation factor [28,29], is higher in Vitro ES cells. Thus, mRNA expression patterns of several methylation-related genes tended to shift, resulting in the promotion of demethylation and the inhibition of methylation in Vitro ES cells. In contrast, at later passages, no significant differences between Vivo and Vitro ES cells were found with respect to the pluripotent marker genes and methylation-related genes that were examined.

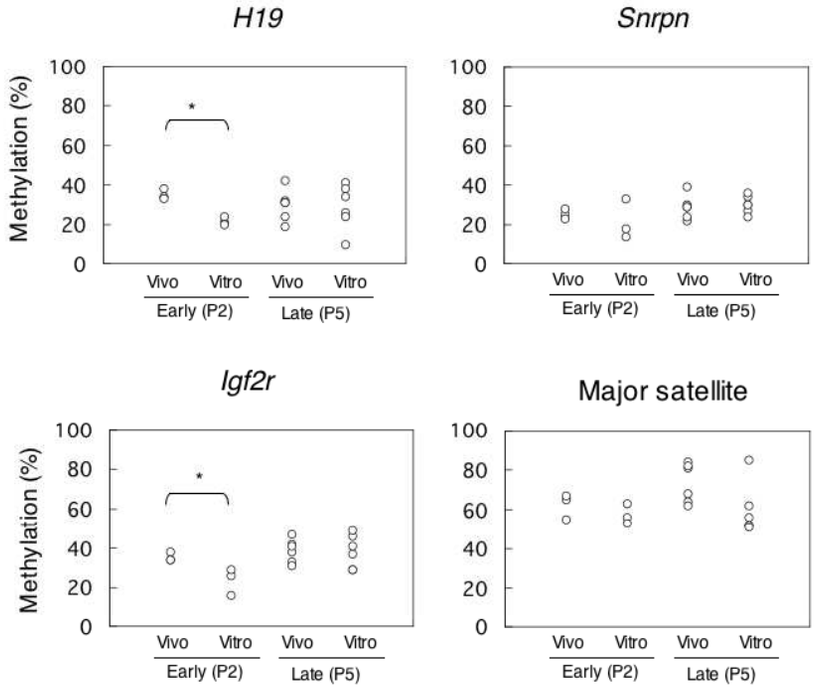

Figure 3. Epigenetic differences between Vivo and Vitro ES cells. DNA methylation status of imprinted genes, *H19*, *Snrpn* and *Igf2r*, and major satellite repeats were examined by COBRA in each ES cell line at an early passage (P2) and a later passage (P5). These graphs summarize previously reported data [1]. *, $P < 0.05$.

7. Epigenetic instability in SCNT and uniparental ES cells

7.1. SCNT ES cells

Maintenance of the normal epigenetic state in SCNT-ES cells is crucial for their use in therapeutic applications. We established two SCNT-ES cell lines from embryos that were produced by introducing mouse embryonic fibroblast (MEF) donor cells into enucleated oocytes. Only two ES cell lines were generated by SCNT, which give a small sample size to examine, but the DNA methylation state of imprinted genes seems to be more severely altered compared to normal ES cell lines at early passages (Fig. 2 and Fig. 4). The abnormal DNA methylation in SCNT-ES cells undergoes further changes during prolonged culture (P10 and P30). For example, the imprinting methylation of the *Snrpn* gene has been completely lost in both the Nt-1 and Nt-2 lines, and that of the *H19* gene has been completely lost in the Nt-1 line (Fig. 4). Chang *et al.* reported that the *H19* imprinted gene displays distinct abnormalities both in SCNT-ES and fertilized embryo-derived ES cell lines after long-term culture *in vitro*, and both exhibit indistinguishable DNA methylation patterns of the imprinted gene [30]. Nevertheless, methylation imprints vary widely in cultured donor cells and their derivative cloned mice, even across the same subclone of donor cells [22]. In fact, results from previous studies indicate that the methylation state of imprinted genes is frequently disrupted in SCNT embryos and their derivative cloned animals [31,32]. In addition, the process of nuclear transfer itself could alter the DNA methylation and gene expression [33]. Thus, the epigenetic marks in SCNT-ES cells may potentially be varied and altered compared to normal ES cells, at least in early passages.

7.2. Uniparental (parthenogenetic) ES cells

We and other groups have suggested that parthenogenetic ES (PgES) cells may be a pluripotent stem cell that could serve as a source of tissue for transplantation [34-36]. PgES cells do not require the destruction of viable biparental embryos as do normal ES cells. In addition, PgES cells do not need viruses or expression plasmids for the establishment of iPS cells. These are very powerful advantages for therapeutic applications. However, the biased epigenetic status and poor pluripotency of parthenogenetic cells are major issues to be overcome. PgES cells are established from parthenogenetic embryos that are produced by the artificial activation of the oocyte. Therefore, PgES cells that possess only maternal genomes could exhibit biallelic or silenced expression of imprinted genes, which causes poor pluripotency. Indeed, parthenogenetic embryos show poor growth and restricted tissue contribution in chimeras [37,38]. However, established PgES cells exhibit an improved contribution to chimeras, compared to chimeras derived from parthenogenetic embryos [39]. Recent reports have shown that loss of imprinting occurred in PgES cells and derivative somatic cells in chimeras and led to changes in the gene expression of imprinted genes and improved pluripotency [2,40]. For example, *Peg1* and *Snrpn* genes are originally silenced in parthenogenetic cells, whereas expression of these genes is elevated in PgES cells by demethylation of the DMR of each gene. PgES cell lines that were reprogrammed by loss of imprinting are closest to normal ES cell lines in

http://dx.doi.org/10.5772/54367

terms of gene expression pattern and pluripotency. Thus, reprogrammed PgES cells will provide a good tool for therapeutic applications. This is a case in which epigenetic instability in ES cells resulted in a desirable outcome. However, epigenetic instability in ES cells most often leads to undesirable results.

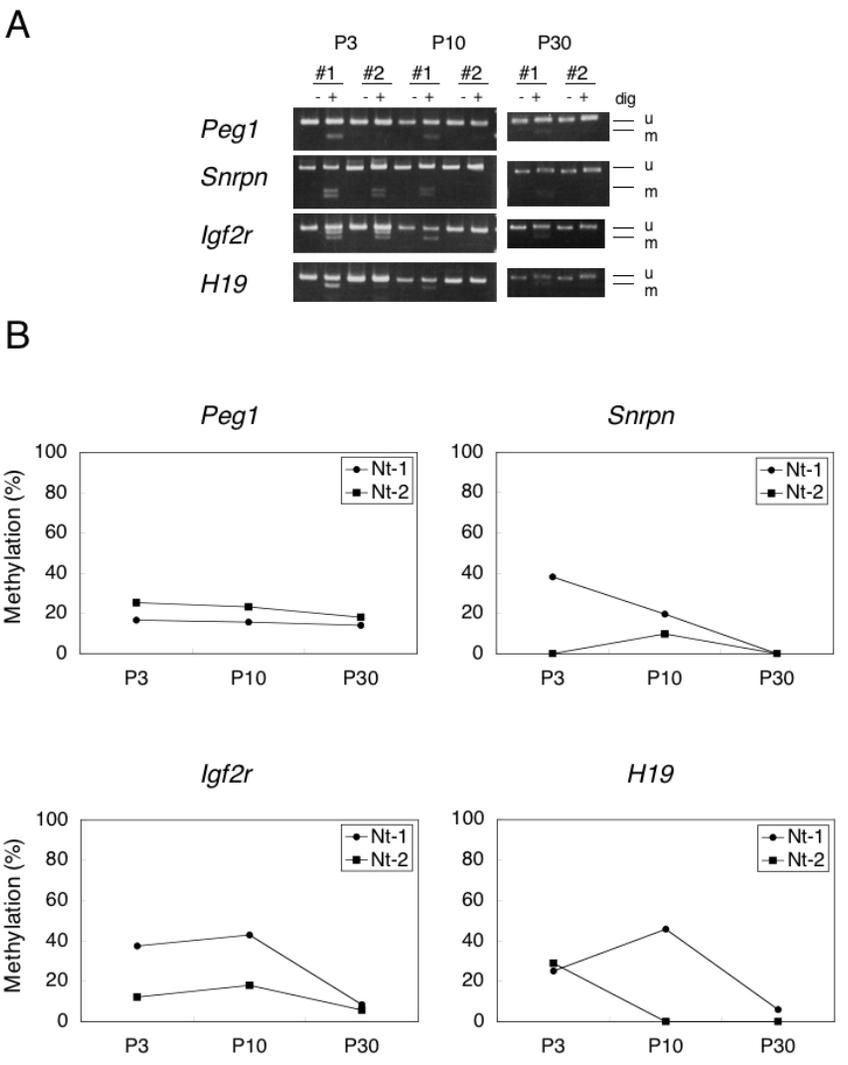

Figure 4. Epigenetic instability in SCNT-ES cells during prolonged culture. Methylation in two SCNT ES cell lines (Nt-1 and Nt-2) was examined by COBRA during prolonged *in vitro* culture (P3, P10 and P30).

8. Effect of altered DNA methylation on pluripotency and disease

In humans, a growing number of reports suggest that children born following ART have an increased risk of developing epigenetic diseases such as Beckwith-Wiedemann syndrome [41,42] and Angelman Syndrome [43], which are caused by epigenetic modifications of im-

printed genes. In sheep, epigenetic changes in the *Igf2r* imprinted gene are associated with fetal overgrowth after IVC [44]. Genome-wide altered DNA methylation also causes epigenetic diseases. For example, genome-wide DNA hypomethylation is commonly observed in human cancers and schizophrenia, and occasionally induces tumors in mice [45-47]. Moreover, hypomethylation in the classical DNA satellites II and III, which are major components of constitutive heterochromatin, is found in ICF (immunodeficiency, centromeric instability, facial anomalies) syndrome in humans [48].

How do these abnormalities in ES cells affect chimeric mice or ES cell-derived tissues? Several studies have indicated that the accumulation of epigenetic alterations during prolonged culture causes a loss of pluripotency in ES cells [21,49]. In chimeras, prolonged culture of ES cells gives rise to abnormalities and frequently results in postnatal death of chimeras possessing a high ES cell contribution [20]. One reason for these problems could be that a loss of imprinting enhances tumorigenesis. In fact, mice derived from ES cells that had a global loss of DNA methylation display widespread cancer formation [50].

9. Candidate genes that cause altered DNA methylation

9.1. DNA methyltransferases

The most important factors for the maintenance of DNA methylation are the DNA methyltransferases. Three CpG DNA methyltransferases, Dnmt1, Dnmt3a and Dnmt3b, coordinately regulate CpG methylation in the genome [12-14]. Deletion of Dnmt1, Dnmt3a or Dnmt3b induces hypomethylation of genomic DNA [14,51], and forced expression of Dnmts causes genomic hypermethylation [52-54]. One of the Dnmt family members, Dnmt3L, is not expressed in differentiated somatic cells but is expressed in ES cells. Although Dnmt3L lacks the functional domains required for catalytic activity, overexpression or downregulation of Dnmt3L results in changes in DNA methylation in ES cells [55]. Thus, the upregulation or downregulation of Dnmts could cause epigenetic instability in ES cells. Indeed, hypomethylation in XX ES cells is associated with reduced levels of Dnmt3a and Dnmt3b, which is the result of both X chromosomes being in the active state [25]. Among Dnmts, a number of alternative splicing variants that lack the regulatory and/or catalytic regions have been reported. In particular, Dnmt3b has nearly 40 different isoforms generated by alternative splicing and/or alternative promoter usage. We recently reported that murine Dnmt3b lacking exon 6 (exon 5 in human) is highly expressed in *in vitro* manipulated embryos and their derivative ES cells that exhibit CpG hypomethylation [17]. Gopalakrishnan *et al*. reported that this isoform is expressed in tumor and iPS cells, and that ectopic overexpression resulted in repetitive element hypomethylation [56]. Similarly, forced expression of human specific DNMT3B4, which lacks a catalytic domain, induced DNA demethylation on satellite 2 in pericentromeric DNA [57]. These reports indicate that Dnmts have complex roles in the maintenance of the DNA methylation state. If this balance collapses, epigenetic instability will result.

http://dx.doi.org/10.5772/54367

9.2. Other methylation factors

Other new methylation factors are Stella (PGC7) and Zfp57. Stella (PGC7), a primordial germ cell and ES cell marker, protects against DNA demethylation in early embryogenesis [58]. Zfp57, a putative KRAB zinc finger protein, is also required for the post-fertilization maintenance of maternal and paternal methylation at multiple imprinted domains [59]. Reductions of the levels of these factors could induce hypomethylation of DNA in ES cells.

9.3. Active demethylation factors

Active DNA demethylation via the base excision repair pathway has recently been proposed in mammals. In zebrafish, the coupling of a deaminase (activation-induced cytidine deaminase, AID), a glycosylase (methyl-CpG binding domain protein 4, MBD4), and Gadd45 is involved in DNA demethylation [60]. In mammals, AID is indeed required for reprogramming of the somatic cell genome by demethylation of pluripotency genes in ES-somatic cell fusion [61]. Gadd45 also promotes epigenetic gene activation by repair-mediated demethylation in mammals [28,29]. A *Gadd45b* gene is activated in Vitro ES cells that possess hypomethylated imprinted genes and repetitive sequences [1]. Another recently proposed demethylation pathway is the conversion of 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC) mediated by the Ten-eleven translocation (TET) proteins, which ultimately results in DNA demethylation [62-63]. In fact, the TET proteins (Tet1 and Tet2) that regulate 5-hmC production [64] are abundantly expressed in ES cells and may be a cause of epigenetic instability in ES cells.

9.4. Chromatin structure specific to ES cells

In ES cells, bivalent domains of chromatin, that regulate several key developmental genes, contain both repressive (histone H3 lysine 27 methylation) and activating (histone H3 lysine 4 methylation) histone modifications that are usually mutually exclusive [65]. Bivalent domains silence developmental genes in ES cells while preserving their potential to become activated upon initiation of specific differentiation programs. DNA methylation was thought to determine the chromatin structure; however, recent reports suggest that chromatin can affect DNA methylation and demethylation [66-67]. Therefore, bivalent chromatin modifications specific to ES cells could be associated with DNA methylation instability.

10. Conclusion

ES cells exhibit instabilities in DNA methylation that are correlated with the origin of the blastocysts from which they were derived (*in vivo*, *in vitro*, SCNT and uniparental), the culture conditions, sex, and prolonged culture. Epigenotyping of ES cells should be adopted as a prerequisite safety evaluation before their use in chimera production or therapeutic applications. Furthermore, genes associated with aberrant DNA methylation should be monitored in ES cell lines to ensure that the cells do not accumulate epigenetic instabilities.

Nomenclature

5-hmC, 5-hydroxymethylcytosine; 5-mC, 5-methylcytosine; AID, activation-induced cytidine deaminase; COBRA, Combined bisulfite restriction analysis; DMR, differentially methylated region; Dnmt, DNA methyltransferase; ES, embryonic stem; Gadd45, Growth arrest and DNA damage-inducible protein 45; ICM, inner cell mass; IVC, *in vitro* culture; IVF, *in vitro* fertilization; MBD4, methyl-CpG binding domain protein 4; PgES, parthenogenetic ES; SCNT, somatic cell nuclear transfer; TET, Ten-eleven translocation; Vitro ES, ES cells derived from in vitro manipulated embryos; Vivo ES, ES cells derived from embryos developed in vivo.

Acknowledgements

We thank Mr. Eikichi Yanagisawa, Dr. Sumiyo Morita, Ms. Mika Kimura, Mr. Daiki Tamura, Mr. Ryohei Kobayashi and Dr. Yasumitsu Nagao for technical assistance and helpful comments on the manuscript. This work was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan; the Ministry of Health, Labour and Welfare of Japan; the National Institute of Biomedical Innovation; and the Takeda Science Foundation.

Author details

Takuro Horii and Izuho Hatada*

*Address all correspondence to: hatada@gunma-u.ac.jp

Biosignal Genome Resource Center, Gunma University, Maebashi, Japan

References

[1] Horii T, Yanagisawa E, Kimura M, Morita S, Hatada I. Epigenetic differences between embryonic stem cells generated from blastocysts developed in vitro and in vivo. Cellular Reprogramming 2010;12(5) 551-63.

[2] Horii T, Kimura M, Morita S, Nagao Y, Hatada I. Loss of genomic imprinting in mouse parthenogenetic embryonic stem cells. Stem Cells 2008;26(1) 79-88.

[3] Antequera F, Bird A. CpG islands in DNA methylation. In: Jost JP, Saluz HP (eds.) Molecular Biology and Biological Significance. Basel: Birkhauser Verlag; 1993. p169-85.

[4] Jaenisch R. DNA methylation and imprinting: why bother? Trends in Genetics 1997;13(8) 323-9.

[5] Jones P, Gonzalgo M. Altered DNA methylation and genome instability: a new pathway to cancer? Proceedings of National Academy Sciences of the United States of America 1997;94(6) 2103-5.

[6] Robertson KD, Wolffe AP. DNA methylation in health and disease. Nature Reviews Genetics 2000;1(1) 11–9.

[7] Surani MA. Imprinting and the initiation of gene silencing in the germline. Cell 1998;93(3) 309-12.

[8] Bartolomei MS, Zemel S, Tilghman SM. Parental imprinting of the mouse H19 gene. Nature 1991;351(6322) 153-5.

[9] Ferguson-Smith AC, Cattanach BM, Barton SC, Beechey CV, Surani MA. Embryological and molecular investigations of parental imprinting on mouse chromosome 7. Nature 1991;351(6328) 667-70.

[10] Pfeifer K. Mechanisms of genomic imprinting. American Journal of Human Genetics. 2000;67(4) 777-87.

[11] Szabo PE, and Mann JR. Biallelic expression of imprinted genes in the mouse germ line: implications for erasure, establishment, and mechanisms of genomic imprinting. Genes and Development 1995;9(15) 1857-68.

[12] Li E, Bestor TH, Jaenisch R. Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. Cell 1992;69(6) 915-26.

[13] Okano M, Xie S, Li E. Cloning and characterization of a family of novel mammalian DNA (cytosine-5) methyltransferases. Nature Genetics 1998;19(3) 219-20.

[14] Okano, M., D. W. Bell, D. A. Haber, and E, Li. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell 1999;99(3) 247-57.

[15] Doherty AS, Mann MR, Tremblay KD, Bartolomei MS, Schultz RM. Differential effects of culture on imprinted H19 expression in the preimplantation mouse embryo. Biology of Reproduction 2000;62(6) 1526-35.

[16] Fernández-Gonzalez R, Moreira P, Bilbao A, Jiménez A, Pérez-Crespo M, Ramírez MA, Rodríguez De Fonseca F, Pintado B, Gutiérrez-Adán A. Long-term effect of in vitro culture of mouse embryos with serum on mRNA expression of imprinting genes, development, and behavior. Proceedings of National Academy Sciences of the United States of America 2004;101(16) 5880-5.

[17] Horii T, Suetake I, Yanagisawa E, Morita S, Kimura M, Nagao Y, Imai H, Tajima S, Hatada I. The Dnmt3b splice variant is specifically expressed in in vitro-manipulated

blastocysts and their derivative ES cells. Journal of Reproduction and Development. 2011;57(5) 579-85.

[18] Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. Nature 1981;292(5819) 154-6.

[19] Martin GR. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proceedings of National Academy Sciences of the United States of America 1981;78(12) 7634-8.

[20] Nagy A, Gócza E, Diaz EM, Prideaux VR, Iványi E, Markkula M, Rossant J. Embryonic stem cells alone are able to support fetal development in the mouse. Development 1990;110(3) 815-21.

[21] Dean W, Bowden L, Aitchison A, Klose J, Moore T, Meneses JJ, Reik W, Feil R. Altered imprinted gene methylation and expression in completely ES cell-derived mouse fetuses: association with aberrant phenotypes. Development 1998;125(12) 2273-82.

[22] Humpherys D, Eggan K, Akutsu H, Hochedlinger K, Rideout WM 3rd, Biniszkiewicz D, Yanagimachi R, Jaenisch R. Epigenetic instability in ES cells and cloned mice. Science 2001;293(5527) 95-7.

[23] Minoguchi S, Iba H. Instability of retroviral DNA methylation in embryonic stem cells. Stem Cells 2008;26(5) 1166-73.

[24] Allegrucci C, Wu YZ, Thurston A, Denning CN, Priddle H, Mummery CL, Ward-van Oostwaard D, Andrews PW, Stojkovic M, Smith N, Parkin T, Jones ME, Warren G, Yu L, Brena RM, Plass C, Young LE. Restriction landmark genome scanning identifies culture-induced DNA methylation instability in the human embryonic stem cell epigenome. Human Molecular Genetics 2007;16(10) 1253-68.

[25] Zvetkova I, Apedaile A, Ramsahoye B, Mermoud JE, Crompton LA, John R, Feil R, Brockdorff N. Global hypomethylation of the genome in XX embryonic stem cells. Nature Genetics 2005;37(11), 1274-9.

[26] Murry CE, Keller G. Differentiation of embryonic stem cells to clinically relevant populations: lessons from embryonic development. Cell 2008;132(4) 661-80.

[27] Mann MR, Lee SS, Doherty AS, Verona RI, Nolen LD, Schultz RM, Bartolomei MS. Selective loss of imprinting in the placenta following preimplantation development in culture. Development 2004;131(15) 3727-35.

[28] Barreto G, Schafer A, Marhold J, Stach D, Swaminathan SK, Handa V, Doderlein G, Maltry N, Wu W, Lyko F, Niehrs C. Gadd45a promotes epigenetic gene activation by repair-mediated DNA demethylation. Nature 2007;445(7128) 671-5.

[29] Ma DK, Jang MH, Guo JU, Kitabatake Y, Chang ML, Pow-Anpongkul N, Flavell RA, Lu B, Ming GL, Song H. Neuronal activity-induced Gadd45b promotes epigenetic DNA demethylation and adult neurogenesis. Science 2009;323(5917) 1074-7.

[30] Chang G, Liu S, Wang F, Zhang Y, Kou Z, Chen D, Gao S. Differential methylation status of imprinted genes in nuclear transfer derived ES (NT-ES) cells. Genomics 2009;93(2) 112-9.

[31] Inoue K, Kohda T, Lee J, Ogonuki N, Mochida K, Noguchi Y, Tanemura K, Kaneko-Ishino T, Ishino F, Ogura A. Faithful expression of imprinted genes in cloned mice. Science 2002;295(5553) 297.

[32] Mann MR, Chung YG, Nolen LD, Verona RI, Latham KE, Bartolomei MS. Disruption of imprinted gene methylation and expression in cloned preimplantation stage mouse embryos. Biology of Reproduction 2003;69(3) 902-14.

[33] Hikichi T, Kohda T, Wakayama S, Ishino F, Wakayama T. Nuclear transfer alters the DNA methylation status of specific genes in fertilized and parthenogenetically activated mouse embryonic stem cells. Stem Cells 2008;26(3) 783-8.

[34] Cibelli JB, Grant KA, Chapman KB, Cunniff K, Worst T, Green HL, Walker SJ, Gutin PH, Vilner L, Tabar V, Dominko T, Kane J, Wettstein PJ, Lanza RP, Studer L, Vrana KE, West MD. Parthenogenetic stem cells in nonhuman primates. Science 2002;295(5556) 819.

[35] Kim K, Lerou P, Yabuuchi A, Lengerke C, Ng K, West J, Kirby A, Daly MJ, Daley GQ. Histocompatible embryonic stem cells by parthenogenesis. Science 2007;315(5811) 482-6.

[36] Horii T, Hatada I. Reprogrammed parthenogenetic ES cells - new choice for regenerative medicine. In: Atwood C.S. (ed.) Methodological Advances in the Culture, Manipulation and Utilization of Embryonic Stem Cells for Basic and Practical Applications. Rejeca: InTech; 2011. p221-36.

[37] Surani, M. A. H. and Barton, S. C. Development of gynogenetic eggs in the mouse: implications for parthenogenetic embryos. Science 1983;222(4627) 1034-1036.

[38] Fundele RH, Norris ML, Barton SC, Fehlau M, Howlett SK, Mills WE, Surani MA. Temporal and spatial selection against parthenogenetic cells during development of fetal chimeras. Development 1990;108(1) 203-11.

[39] Allen ND, Barton SC, Hilton K, Norris ML, Surani MA. A functional analysis of imprinting in parthenogenetic embryonic stem cells. Development 1994;120(6) 1473-82.

[40] Chen Z, Liu Z, Huang J, Amano T, Li C, Cao S, Wu C, Liu B, Zhou L, Carter MG, Keefe DL, Yang X, Liu L. Birth of parthenote mice directly from parthenogenetic embryonic stem cells. Stem Cells 2009;27(9) 2136-45.

[41] Maher ER, Brueton LA, Bowdin SC, Luharia A, Cooper W, Cole TR, Macdonald F, Sampson JR, Barratt CL, Reik W, Hawkins MM. Beckwith-Wiedemann syndrome

and assisted reproduction technology (ART). Journal of Medical Genetics 2003;40(1) 62-4.

[42] DeBaun MR, Niemitz EL, Feinberg AP. Association of in vitro fertilization with Beckwith-Wiedemann syndrome and epigenetic alterations of LIT1 and H19. American Journal of Human Genetics 2003;72(1) 156-60.

[43] Cox GF, Burger J, Lip V, Mau UA, Sperling K, Wu BL, Horsthemke B. Intracytoplasmic sperm injection may increase the risk of imprinting defects. American Journal of Human Genetics 2002;71(1) 162-164.

[44] Young LE, Fernandes K, McEvoy TG, Butterwith SC, Gutierrez CG, Carolan C, Broadbent PJ, Robinson JJ, Wilmut I, Sinclair KD. Epigenetic change in IGF2R is associated with fetal overgrowth after sheep embryo culture. Nature Genetics 2001;27(2) 153-4.

[45] Eden A, Gaudet F, Waghmare A, Jaenisch R. Chromosomal instability and tumors promoted by DNA hypomethylation. Science 2003;300(5618) 455.

[46] Howard G, Eiges R, Gaudet F, Jaenisch R, Eden A. Activation and transposition of endogenous retroviral elements in hypomethylation induced tumors in mice. Oncogene 2008;27(3) 404-8.

[47] Shimabukuro M, Sasaki T, Imamura A, Tsujita T, Fuke C, Umekage T, Tochigi M, Hiramatsu K, Miyazaki T, Oda T, Sugimoto J, Jinno Y, Okazaki Y. Global hypomethylation of peripheral leukocyte DNA in male patients with schizophrenia: a potential link between epigenetics and schizophrenia. Jounal of Psychiatric Research 2007;41(12) 1042-6.

[48] Jeanpierre M, Turleau C, Aurias A, Prieur M, Ledeist F, Fischer A, Viegas-Pequignot E. An embryonic-like methylation pattern of classical satellite DNA is observed in ICF syndrome. Human Molecular Genetics 1993;2(6) 731-5.

[49] Nagy A, Rossant J, Nagy R, Abramow-Newerly W, Roder JC. Derivation of completely cell culture-derived mice from early-passage embryonic stem cells. Proceedings of National Academy Sciences of the United States of America 1993;90(18) 8424-8.

[50] Holm TM, Jackson-Grusby L, Brambrink T, Yamada Y, Rideout WM 3rd, Jaenisch R. Global loss of imprinting leads to widespread tumorigenesis in adult mice. Cancer Cell 2005;8(4) 275-85.

[51] Li E, Beard C, Jaenisch R. Role for DNA methylation in genomic imprinting. Nature 1993;366(6453) 362-5.

[52] Vertino PM, Yen RW, Gao J, Baylin SB. De novo methylation of CpG island sequences in human fibroblasts overexpressing DNA (cytosine-5-)-methyltransferase. Molecular and Cellular Biology 1996;16(8) 4555-65.

[53] Hsieh CL. In vivo activity of murine de novo methyltransferases, Dnmt3a and Dnmt3b. Molecular and Cellular Biology 1999;19(12) 8211-8.

[54] Biniszkiewicz D, Gribnau J, Ramsahoye B, Gaudet F, Eggan K, Humpherys D, Mastrangelo MA, Jun Z, Walter J, Jaenisch R. Dnmt1 overexpression causes genomic hypermethylation, loss of imprinting, and embryonic lethality. Molecular and Cellular Biology 2002;22(7) 2124-35.

[55] Ooi SK, Wolf D, Hartung O, Agarwal S, Daley GQ, Goff SP, Bestor TH. Dynamic instability of genomic methylation patterns in pluripotent stem cells. Epigenetics and Chromatin 2010;3(1) 17.

[56] Gopalakrishnan S, Van Emburgh BO, Shan J, Su Z, Fields CR, Vieweg J, Hamazaki T, Schwartz PH, Terada N, Robertson KD. A novel DNMT3B splice variant expressed in tumor and pluripotent cells modulates genomic DNA methylation patterns and displays altered DNA binding. Molecular Cancer Research 2009;7(10) 1622-34.

[57] Saito Y, Kanai Y, Sakamoto M, Saito H, Ishii H, Hirohashi S. Overexpression of a splice variant of DNA methyltransferase 3b, DNMT3b4, associated with DNA hypomethylation on pericentromeric satellite regions during human hepatocarcinogenesis. Proceedings of National Academy Sciences of the United States of America 2002;99(15) 10060-5.

[58] Nakamura T, Arai Y, Umehara H, Masuhara M, Kimura T, Taniguchi H, Sekimoto T, Ikawa M, Yoneda Y, Okabe M, Tanaka S, Shiota K, Nakano T. PGC7/Stella protects against DNA demethylation in early embryogenesis. Nature Cell Biology 2007;9(1) 64-71.

[59] Li X, Ito M, Zhou F, Youngson N, Zuo X, Leder P, Ferguson-Smith AC. A maternal-zygotic effect gene, Zfp57, maintains both maternal and paternal imprints. Developmental Cell 2008;15(4) 547-57.

[60] Rai K, Huggins IJ, James SR, Karpf AR, Jones DA, Cairns BR. DNA demethylation in zebrafish involves the coupling of a deaminase, a glycosylase, and gadd45. Cell 2008;135(7) 1201-12.

[61] Bhutani N, Brady JJ, Damian M, Sacco A, Corbel SY, Blau HM. Reprogramming towards pluripotency requires AID-dependent DNA demethylation. Nature 2010;463(7284) 1042-7.

[62] Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, Agarwal S, Iyer LM, Liu DR, Aravind L, Rao A. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science 2009;324(5929) 930-5.

[63] Ito S, D'Alessio AC, Taranova OV, Hong K, Sowers LC, Zhang Y. Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. Nature 2010;466(7310) 1129-33.

[64] Koh KP, Yabuuchi A, Rao S, Huang Y, Cunniff K, Nardone J, Laiho A, Tahiliani M, Sommer CA, Mostoslavsky G, Lahesmaa R, Orkin SH, Rodig SJ, Daley GQ, Rao A.

Tet1 and Tet2 regulate 5-hydroxymethylcytosine production and cell lineage specification in mouse embryonic stem cells. Cell Stem Cell 2011;8(2) 200-13.

[65] Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, Fry B, Meissner A, Wernig M, Plath K, Jaenisch R, Wagschal A, Feil R, Schreiber SL, Lander ES. A bivalent chromatin structure marks key developmental genes in embryonic stem cells. Cell 2006;125(2) 315-26.

[66] Tamaru H, Selker EU. A histone H3 methyltransferase controls DNA methylation in Neurospora crassa. Nature 2001;414(6861) 277-83.

[67] Jackson JP, Lindroth AM, Cao X, Jacobsen SE. Control of CpNpG DNA methylation by the KRYPTONITE histone H3 methyltransferase. Nature 2002;416(6880) 556-60.

[68] Bachman KE, Park BH, Rhee I, Rajagopalan H, Herman JG, Baylin SB, Kinzler KW, Vogelstein B. Histone modifications and silencing prior to DNA methylation of a tumor suppressor gene. Cancer Cell 2003;3(1) 89-95.