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BRIEF ARTICLE

Epigenetic states and expression of imprinted genes in human embryonic stem cells

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Abstract

AIM: To investigate the epigenetic states and expression of imprinted genes in five human embryonic stem cell (hESC) lines derived in Taiwan.

METHODS: The heterozygous alleles of single nucleotide polymorphisms (SNPs) at imprinted genes were analyzed by sequencing genomic DNAs of hESC lines and the monoallelic expression of the imprinted genes were confirmed by sequencing the cDNAs. The expression profiles of 32 known imprinted genes of five hESC lines were determined using Affymetrix human genome U133 plus 2.0 DNA microarray.

RESULTS: The heterozygous alleles of SNPs at seven imprinted genes, *IPW*, *PEG10*, *NESP55*, *KCNQ1*, *ATP10A*, *TCEB3C* and *IGF2*, were identified and the monoallelic expression of these imprinted genes except *IGF2* were confirmed. The *IGF2* gene was found to be imprinted in hESC line T2 but partially imprinted in line T3 and not imprinted in line T4 embryoid bodies. Ten imprinted genes, namely *GRB10*, *PEG10*, *SGCE*, *MEST*, *SDHD*, *SNRPN*, *SNURF*, *NDN*, *IPW* and *NESP55*, were found to be highly expressed in the undifferentiated hESC lines and down-regulated in differentiated derivatives. The *UBE3A* gene abundantly expressed in undifferentiated hESC lines and further up-regulated in differentiated tissues. The expression levels of other 21 imprinted genes were relatively low in undifferentiated hESC lines and five of these genes (*TP73*, *COPG2*, *OSBPL5*, *IGF2* and *ATP10A*) were found to be up-regulated in differentiated tissues.

CONCLUSION: The epigenetic states and expression of imprinted genes in hESC lines should be thoroughly studied after extended culture and upon differentiation in order to understand epigenetic stability in hESC lines before their clinical applications.

Key words: DNA microarray; Imprinting; Single nucleotide polymorphism; Human embryonic stem cell

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INTRODUCTION

Genomic imprinting, the parent-of-origin-specific silenc-

ing of genes, is an epigenetic modification that gives rise to differential expression of paternally and maternally inherited alleles of some genes. The imprinting is established afresh in the germ line in each generation and stably inherited throughout the somatic cell division[1-3]. Imprinted genes play important roles in human fetal and placental development and aberrant expression of imprinted genes is associated with human diseases including several cancers and a number of neurological disorders such as Prader-Willi syndrome[4-8].

Human embryonic stem cell (hESC) lines were derived from inner cell mass of blastocysts produced by *in vitro* fertilization using mitotically inactivated mouse embryonic fibroblast cells as feeder layer[9]. Thus far, many hESC lines have been derived and characterized (http://www.nih.gov/news/stemcell/). Because of the dual abilities to proliferate indefinitely and differentiate into various cell type derivatives of all three embryonic germ layers, ectoderm, mesoderm and endoderm, the hESC lines could potentially provide an unlimited supply of different cell types for transplantation therapy to treat a variety of degenerative diseases such as Parkinson's disease, spinal cord injury, diabetes and heart failure[10-13].

In vitro fertilization has been reported to increase human diseases caused by aberrant genomic imprinting[14] and abnormal imprinting has also been reported in mouse embryonic stem cells[15]. Furthermore, a large number of the imprinting genes show discordance of their imprinting states between human and mouse[16]. Therefore, it is important to monitor and maintain epigenetic stabilities in hESC lines for transplantation purposes[13]. However, little is known about the epigenetic states and the expression profiles of imprinted genes in hESC lines following extended culture and upon differentiation. In the present study, the allele-specific expressions of seven imprinted genes in five hESC lines derived in Taiwan[17] were investigated using single nucleotide polymorphism (SNP) markers. In addition, the expression profiles of 32 known imprinted genes[16] in undifferentiated state and some of differentiated derivatives of these five hESC lines were analyzed using DNA microarray.

MATERIALS AND METHODS

Allele-specific expression of imprinted genes

Genomic DNAs (gDNA) were isolated using the Wizard SV Genomic DNA Purification System (Promega) and total RNAs were extracted using the Absolutely RNA Nanoprep Kit (Stratagene) from undifferentiated cells, embryoid bodies and/or teratomas of hESC lines. The cDNAs were synthesized using the 'Microarray' Target Amplification Kit and purified with 'Microarray' Target Purification Kit (Roche Applied Science). Polymerase chain reaction (PCR) amplification of genomic DNA and cDNA was carried out in a 25 uL reaction volume with 2 units of the Go Tag Flexi DNA polymerase (Promega), 1x supplied reaction buffer, 0.12 umol/L of each primer, 0.75 mmol/L $MgCl_2$, 0.2 mmol/L of dNTPs and 10-200 ng DNA template. Cycle conditions are as follows: initial denaturation at 95℃ for 2 min then 30 cycles of 95℃ for 30 s, 55℃ for 30 s and 72℃ for 3 min followed by a final extension at 72℃ for 5 min. Primer sequences are given in Table 1. Amplified DNA was purified using the Wizard SV Gel and PCR Clean-up System (Promega) and sequenced with the BigDye terminator cycle Sequencing Kit (3.1 version) and ABI 3730 DNA sequencer.

Expression profiling of imprinted genes in human embryonic stem cell lines

Five hESC lines were derived with IRB approval from surplus blastocysts in Taiwan and continuously cultured on mitotically inactivated mouse embryonic fibroblast feeder layer for more than 44 passages. hESC lines T1 and T3 possess normal female karyotypes whereas lines T4 and T5 are normal male but line T2 is male trisomy 12 (47XY, + 12). hESC lines T1, T2, T3 and T5 were able to produce teratomas in SCID mice and line T4 could only form embryoid bodies *in vitro*. Expression profiles of imprinted genes from undifferentiated hESC lines, embryoid bodies and teratoma were analyzed using Affymetrix human genome U133 plus 2.0 GeneChip containing 54 675 probe sets for 47 400 transcripts and variants including 38 500 well-characterized human genes, as previously reported[17]. It may be noted that Affymetrix GeneChip expression analysis can be used as a stand-alone quantitative comparison since the correlation between Affymetrix GeneChip results and TagMan RT-qPCR results was shown in a good linearity of $R^2 = 0.95$ by the MicroArray Quality Control Study, a collaborative effort of 137 scientists led by the US-FDA[18,19].

RESULTS

Allele-specific expression of imprinted genes

In order to distinguish mRNA transcripts from each parental allele, the potential SNPs of the 32 known imprinted genes[16] were researched from the literature[20,21] and SNP database of NCBI. The heterozygous alleles of SNPs at seven genes, *IPW*, *PEG10*, *NESP55*, *KCNQ1*, *ATP10A*, *TCEB3C* and *IGF2* genes, were found by sequencing gDNA of hESC lines (Table 2). The gDNA of hESC lines T1 and T2 exhibited T and C alleles of *IPW* gene whereas the cDNA sequencing of undifferentiated cells and teratomas from hESC lines T1 and T2 showed only T allele of *IPW* gene (Figure 1). The genomic DNA from hESC lines T2 and T3 exhibited C and T alleles of *PEG10* gene whereas the cDNA sequencing of undifferentiated hESC T2 and T3 cells, as well as hESC line T2 teratoma, showed only C allele of *PEG10* gene. The T and C alleles of *NESP55* gene were identified in the genomic DNA of hESC line T1 whereas the cDNA from hESC line T1 teratoma (TT1) showed only T allele of *NESP55* gene. The G and A alleles of *KCNQ1* gene were identified in genomic DNA of hESC line T3 whereas only G allele of *KCNQ1* was found in the sequencing cDNA from undifferentiated hESC line T3 cells. The C and G alleles of *ATP10A* gene

Table 1 Polymerase chain reaction primer sequences and polymorphisms

Gene	Primer sequence (5'→3')	Size (bp)	SNP	Acc. No Location	Ref.
IGF2	F CTTGGACTTTGAGTCAAATTGG	235	G→A	X07868	[18]
	R CCTCCTTTGGTCTTACTGGG			Pos. 820	
IPW	F GGGAACTCTTCTGGGAGTGAATGTTATCA	1550	C→T	U12897	[18]
	R GGGAGGTTCATTGCACAGAAATTTGG			Pos. 1670	
	Seq. TGGATAGATGCACACAAACAC	868			
NESP55 gDNA	F GGCTCCTTGTGCTGTCTGTCTTGTAG	233	T→C	M21741	[18]
	R CCACACAAGTCGGGGTGTAGCTTA			Pos. 299	
NESP55 cDNA	F TCGGAATCTGACCACGCGCA	1141			
	R CACGAAGATGATGGCAGTCAC				
	Seq. CAACCTGAAAGAGGCGATTGAA				
PEG10	F TCATTTTCCTGCCTGGTTGC	405	C→T	XM_496907	[19]
	R GGAGCCTCTCATTCACAGC			Pos. 4404	
KCNQ1 gDNA	F CACTGCCTGCACTTTGAGCC	282	G→A	AJ006345	[19]
	R GTGAGGAGAAGGGGGTGGTT			Pos. 331010	
KCNQ1 cDNA	F GGACCTGGAAGGGGAGACT	282			
	R GCGATCCTTGCTCTTTTCT				
ATP10A	F AAAGACACCACCGACAGGAA	318	G→C	BC052251	This study
	R ATGCTCATGTCCACTGTGCT			Pos. 4006	
TCEB3C	F CCAGAGCTGAGAGAAAGTGC	249	C→G	NM_145653	This study
	R TTTCCTGGCGAGACGATTTG			Pos. 772	

gDNA: Genomic DNA; SNP: Single nucleotide polymorphism.

were found in the genomic DNA of hESC line T2 whereas the sequencing cDNA products from undifferentiated cells and teratoma of hESC line T2 showed only C allele of *ATP10A* gene. The G and C alleles of *TCEB3C* gene were found in the gDNA of hESC lines T1 whereas only G allele of *TCEB3C* gene was identified in the cDNAs of hESC line TT1. These results clearly demonstrated the monoallelic expression of six imprinted genes, *IPW*, *PEG10*, *NESP55*, *KCNQ1*, *ATP10A* and *TCEB3C,* in undifferentiated hESC lines and/or differentiated derivatives. As to *IGF2* gene, the A and G alleles were identified by sequencing genomic DNA of hESC lines T2 and T3 whereas the cDNA sequencing of undifferentiated cells and teratoma from hESC line T2 showed only A allele. However, the cDNA of undifferentiated cells from hESC line T3 detected the full expression of G allele and partial expression of A allele, indicating the partially relaxed imprinting of *IGF2* gene. Furthermore, the embryoid bodies of hESC line T4 (EB4) showed equal expression of both A and G alleles, indicating no imprinting of *IGF2* gene.

Expression profiles of imprinted genes in human embryonic stem cell lines

Expression profiles of the 32 known imprinted genes[16] from five undifferentiated hESC lines, T4 embryoid bodies (EB4) and TT1 were analyzed using Affymetrix human genome U133 plus 2.0 GeneChip and the results are given in Table 3. Ten imprinted genes, namely *GRB10*, *PEG10*, *SGCE*, *MEST*, *SDHD*, *SNRPN*, *SNURF*, *NDN*, *IPW* and *NESP55*, were found to be highly expressed in the undifferentiated hESC lines and down-regulated in differentiated derivatives (EB4 and TT1) (Table 3 top). The UBE3A gene abundantly expressed in undifferentiated hESC lines and further up-regulated in differentiated tissues (EB4 and TT1). The expression levels of other 21 imprinted genes were relatively low in undifferentiated hESC lines and six of them (*TP73*, *COPG2*, *OSBPL5*, *IGF2*, *ATP10A* and *PEG*) were found to be up-regulated in differentiated tissues (EB4 and TT1) (Table 3 bottom).

Table 2 Allele-specific expression of imprinted genes in human embryonic stem cell lines

Genes	hESC lines	gDNA	cDNA		
			Undiff. cells	Tetratoma	EB
IPW	T1	T/C	T	T	
	T2	T/C	T	T	
PEG10	T2	C/T	C	C	
	T3	C/T	C		
NESP55	T1	T/C		T	
KCNQ1	T3	G/A	G		
ATP10A	T2	C/G	C	C	
TCEB3C	T1	G/C		G	
IGF2	T2	A/G	A	A	
	T3	G/A	G > A[a]		
	T4				A/G[b]

[a]Indicates partially expressed A allele; [b]Denotes bi-allelic expression. gDNA: Genomic DNA; EB: Embryoid bodies.

DISCUSSION

The five hESC lines were previously derived with IRB approval in Taiwan and hESC lines T1, T 2, T3 and T5 were able to produce teratomas in SCID mice while hESC line T4 could only form embryoid bodies *in vitro*[17]. In this investigation, the monoallelic expression of six imprinted genes (*IPW*, *PEG10*, *NESP55*, *KCNQ1*, *ATP10A* and *TCEB3C*) was confirmed in undifferentiated hESC lines and/or differentiated teratomas. The monoallelic expression of *PEG10*, *NESP55* and *KCNQ1* genes was also reported previously in hESC lines[20,21]. However, the IGF2

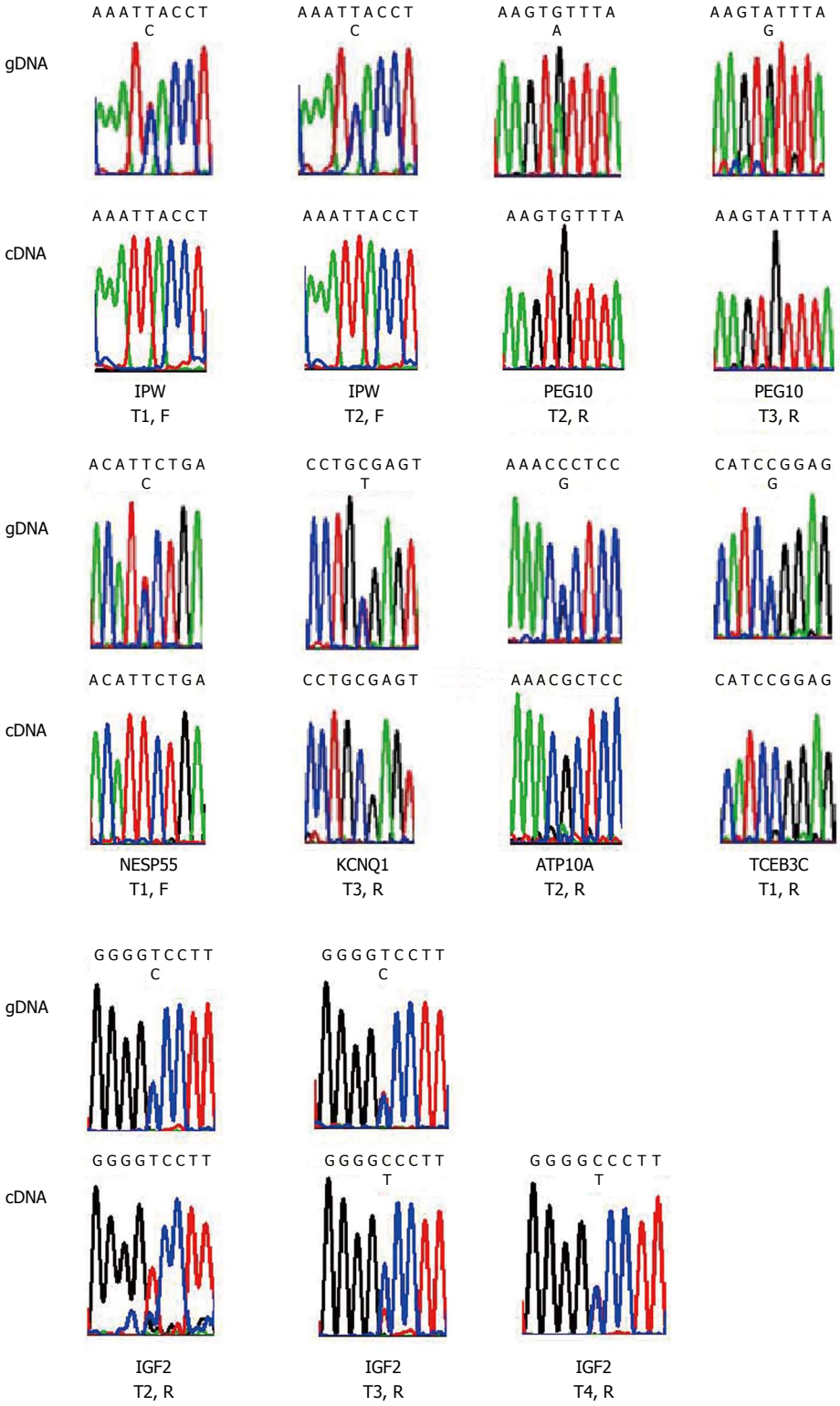

Figure 1 Allele-specific expression of seven imprinted genes. The heterozygous alleles of single nucleotide polymorphism (SNPs) at seven genes, *IPW*, *PEG10*, *NESP55*, *KCNQ1*, *ATP10A*, *TCEB3C* and *IGF2* genes, were found by sequencing genomic DNAs (gDNA) of hESC lines T1, T2, T3 and T4 (Table 2). The cDNA sequences of undifferentiated cells and teratomas from hESC lines T1, T2 and/or T3, as well as embryoid bodies of hESC line T4, were determined using either forward (F) or reverse (R) primers given in Table 1. It may be noted that the peak height of heterozygous alleles (nucleotides) was lower than that of homozygous allele (nucleotide) in addition to two different colors instead of single color. When the non-informative homozygotes at SNPs were detected in gDNA, no cDNA sequencing was carried out.

gene was found to be imprinted in hESC line T2 but partially imprinted in hESC line T3 and not imprinted in hESC line EB4. The different extents of *IGF2* imprinting among different hESC lines might be due to different developmental stages of blastocysts at which hESC lines were derived. The molecular mechanism responsible for

Table 3 Expression of 32 human imprinted genes

Genes	T1	T2	T3	T4	T5	EB4	TT1	Probe ID	Chromosome	Exp. allele
GRB10	2543	3249	3128	3778	5927	68	96	209409_at	7p12-p11.2	P/M
PEG10	1602	1080	1710	2033	3255	1330	683	212094_at	7q21	P
SGCE	1256	1620	1725	751	501	250	737	204688_at	7q21-q22	P
MEST	7066	7416	2609	8727	581	48	28	202016_at	7q32	P
SDHD	2702	4707	2324	4849	4079	104	235	202026_at	11q23	P
SNRPN	4819	3228	6721	5534	5934	2895	1571	228370_at	15q11.2	P
SNURF	42361	58257	27211	64033	57468	11	159	201522_x_at	15q11.2-q12	P
NDN	458	932	734	734	334	8	49	209550_at	15q11.2-q12	P
IPW	2653	2848	1842	4685	8969	113	328	213447_at	15q11-q12	P
GNAS-NESP55	30303	49688	45911	55643	46465	66	905	212273_x_at	20q13.3	M
UBE3A	4119	2012	3125	2362	4491	7812	6646	211285_s_at	15q11-q13	M
HYMAI	60	196	76	16	110	113	401	215513_at	6q24	P
PLAGL1	35	12	22	5	18	79	235	1559282_at	6q24-q25	P
WT1	90	25	56	9	126	31	129	206067_s_at	11p13	P
KCNQ1DN	89	78	31	17	62	15	13	220629_at	11p15.4	M
KCNQ1	232	552	25	170	28	89	159	204487_s_at	11p15.5	M
SLC22A18	274	31	39	519	72	13	134	204981_at	11p15.5	M
PHLDA2	205	131	149	189	131	66	117	209802_at	11p15.5	M
H19	83	100	22	95	12	3	107	224997_x_at	11p15.5	M
CDKN1C	22	30	17	125	19	119	938	213183_s_at	11p15.5	M
DLK1	13	461	38	1049	8	23	32	209560_s_at	14q32	P
MEG3	38	123	215	87	12	40	10	226211_at	14q32	M
HBII-437	97	101	60	128	168	25	1091	214834_at	15q11.2-q12	P
MAGEL2	22	26	7	22	17	40	125	219894_at	15q11-q12	P
MKRN3	82	71	55	168	148	85	304	206585_at	15q11-q13	P
TCEB3C	15	135	13	5	144	49	548	1552860_at	18q21.1	M
TP73	136	62	13	62	78	1241	1058	232546_at	1p36.3	M
COPG2	50	75	15	45	106	1040	737	222298_at	7q32	P
OSBPL5	240	168	170	559	526	1210	1895	233734_s_at	11p15.4	M
IGF2	20	17	66	12	20	2596	2014	210881_s_at	11p15.5	P
ATP10A	138	248	105	198	98	134	1081	214256_at	15q11.2	M
PEG3	72	44	57	14	134	808	479	209242_at	19q13.4	P

EB4: T4 Embryoid bodies; TT1: T1 teratoma. Expression of either maternal (M) or paternal (P) allele was according to Morison *et al*[16] (2005).

this variability of imprinting remains to be elucidated. The *IGF2*, as well as H19 in the same chromosomal region 11P15.5, was also reported to be more variable and thus could potentially provide a sensitive indication of epigenetic status of hESC lines[22]. The *IGF2* gene was also shown to be only partially imprinted in human germ cell-derived lines[23]. It may be noted that the *IGF2* gene was found to be highly expressed in differentiated derivatives, namely hESC lines EB4 and TT1 (see EB4 and TT1 in Table 3).

The expression of imprinted genes plays important roles during early embryo development[6,8]. hESC lines and their differentiated derivatives offer an opportunity for studying the expression of different imprinted genes shortly before and after the embryonic implantation. In this investigation, using DNA microarray we analyzed the expression profiles of 32 known imprinted genes[16] in five undifferentiated hESC lines derived in Taiwan and some of their differentiated derivatives. The expression levels of these 32 imprinted genes were relatively consistent among five hESC lines. It may be noted that five (*SNRPN*, *SNURF*, *NDN*, *IPW* and *UBE3A*) of eleven highly expressed imprinted genes in undifferentiated hESC lines are located on chromosomal region 15q11-q13 (Table 3) and that abnormal expression of *SNRPN* and *NDN* genes results in the neurogenetic disorder known as Prader-Willi Syndrome[5]. In short, the epigenetic states and expression of imprinted genes in hESC lines should be thoroughly studied after extended culture and upon differentiation in order to understand epigenetic stability in hESC lines before their clinical applications[24].

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COMMENTS

Background

Human embryonic stem cell (hESC) lines possess the dual abilities to proliferate indefinitely and differentiate into various cell types in the body. Thus, hESC lines could potentially provide an unlimited supply of different cell types for transplantation therapy. Genomic imprinting is established afresh in the germ cells in each generation and stably inherited throughout the somatic cell divisions.

Research frontiers

The imprinted genes play important roles in human fetal and placental development and aberrant expression of imprinted genes is associated with human diseases. Therefore, it is important to monitor and maintain epigenetic

stabilities in hESC lines before their clinical applications.

Innovations and breakthroughs

Six of seven imprinting genes were shown to be fully imprinted but the extent of *IGF2* imprinting was found to be varied between different hESC lines. The observed variability of *IGF2* imprinting adds to the overall picture of genomic stability of imprinting genes among hESC lines. The *IGF2* gene was further found to be highly expressed in differentiated derivatives.

Applications

The *IGF2* could potentially provide a sensitive indication of epigenetic status of hESC lines. The epigenetic stability of hESC lines should be fully understood before their medical applications.

Terminology

Genomic imprinting: genomic imprinting is an epigenetic modification that gives rise to differential expression of paternally and maternally inherited alleles of some genes. hESC lines: hESC lines were derived from inner cell mass of blastocysts produced by *in vitro* fertilization.

Peer review

Imprinting and epigenetic stability of hESCs is an important issue in the field and, as such, the research performed is important. Although imprinting has previously been studied in hESCs, the observed variability between different hESC lines adds to the overall picture of variations in imprinting amongst hESC lines.

REFERENCES

1 **Bartolomei MS**, Tilghman SM. Genomic imprinting in mammals. *Annu Rev Genet* 1997; **31**: 493-525
2 **Reik W**, Dean W, Walter J. Epigenetic reprogramming in mammalian development. *Science* 2001; **293**: 1089-1093
3 **Haig D**. Genomic imprinting and kinship: how good is the evidence? *Annu Rev Genet* 2004; **38**: 553-585
4 **Maher ER**, Reik W. Beckwith-Wiedemann syndrome: imprinting in clusters revisited. *J Clin Invest* 2000; **105**: 247-252
5 **Nicholls RD**, Knepper JL. Genome organization, function, and imprinting in Prader-Willi and Angelman syndromes. *Annu Rev Genomics Hum Genet* 2001; **2**: 153-175
6 **Verona RI**, Mann MR, Bartolomei MS. Genomic imprinting: intricacies of epigenetic regulation in clusters. *Annu Rev Cell Dev Biol* 2003; **19**: 237-259
7 **Delaval K**, Feil R. Epigenetic regulation of mammalian genomic imprinting. *Curr Opin Genet Dev* 2004; **14**: 188-195
8 **Morgan HD**, Santos F, Green K, Dean W, Reik W. Epigenetic reprogramming in mammals. *Hum Mol Genet* 2005; **14** Spec No 1: R47-R58
9 **Thomson JA**, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. *Science* 1998; **282**: 1145-1147
10 **Reubinoff BE**, Pera MF, Fong CY, Trounson A, Bongso A. Embryonic stem cell lines from human blastocysts: somatic differentiation in vitro. *Nat Biotechnol* 2000; **18**: 399-404
11 **Odorico JS**, Kaufman DS, Thomson JA. Multilineage differentiation from human embryonic stem cell lines. *Stem Cells* 2001; **19**: 193-204
12 **Heins N**, Englund MC, Sjöblom C, Dahl U, Tonning A, Bergh C, Lindahl A, Hanson C, Semb H. Derivation, characterization, and differentiation of human embryonic stem cells. *Stem Cells* 2004; **22**: 367-376
13 **Wobus AM**, Boheler KR. Embryonic stem cells: prospects for developmental biology and cell therapy. *Physiol Rev* 2005; **85**: 635-678
14 **Maher ER**, Afnan M, Barratt CL. Epigenetic risks related to assisted reproductive technologies: epigenetics, imprinting, ART and icebergs? *Hum Reprod* 2003; **18**: 2508-2511
15 **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**: 95-97
16 **Morison IM**, Ramsay JP, Spencer HG. A census of mammalian imprinting. *Trends Genet* 2005; **21**: 457-465
17 **Li SS**, Liu YH, Tseng CN, Chung TL, Lee TY, Singh S. Characterization and gene expression profiling of five new human embryonic stem cell lines derived in Taiwan. *Stem Cells Dev* 2006; **15**: 532-555
18 **Canales RD**, Luo Y, Willey JC, Austermiller B, Barbacioru CC, Boysen C, Hunkapiller K, Jensen RV, Knight CR, Lee KY, Ma Y, Maqsodi B, Papallo A, Peters EH, Poulter K, Ruppel PL, Samaha RR, Shi L, Yang W, Zhang L, Goodsaid FM. Evaluation of DNA microarray results with quantitative gene expression platforms. *Nat Biotechnol* 2006; **24**: 1115-1122
19 **Shi L**, Reid LH, Jones WD, Shippy R, Warrington JA, Baker SC, Collins PJ, de Longueville F, Kawasaki ES, Lee KY, Luo Y, Sun YA, Willey JC, Setterquist RA, Fischer GM, Tong W, Dragan YP, Dix DJ, Frueh FW, Goodsaid FM, Herman D, Jensen RV, Johnson CD, Lobenhofer EK, Puri RK, Schrf U, Thierry-Mieg J, Wang C, Wilson M, Wolber PK, Zhang L, Amur S, Bao W, Barbacioru CC, Lucas AB, Bertholet V, Boysen C, Bromley B, Brown D, Brunner A, Canales R, Cao XM, Cebula TA, Chen JJ, Cheng J, Chu TM, Chudin E, Corson J, Corton JC, Croner LJ, Davies C, Davison TS, Delenstarr G, Deng X, Dorris D, Eklund AC, Fan XH, Fang H, Fulmer-Smentek S, Fuscoe JC, Gallagher K, Ge W, Guo L, Guo X, Hager J, Haje PK, Han J, Han T, Harbottle HC, Harris SC, Hatchwell E, Hauser CA, Hester S, Hong H, Hurban P, Jackson SA, Ji H, Knight CR, Kuo WP, LeClerc JE, Levy S, Li QZ, Liu C, Liu Y, Lombardi MJ, Ma Y, Magnuson SR, Maqsodi B, McDaniel T, Mei N, Myklebost O, Ning B, Novoradovskaya N, Orr MS, Osborn TW, Papallo A, Patterson TA, Perkins RG, Peters EH, Peterson R, Philips KL, Pine PS, Pusztai L, Qian F, Ren H, Rosen M, Rosenzweig BA, Samaha RR, Schena M, Schroth GP, Shchegrova S, Smith DD, Staedtler F, Su Z, Sun H, Szallasi Z, Tezak Z, Thierry-Mieg D, Thompson KL, Tikhonova I, Turpaz Y, Vallanat B, Van C, Walker SJ, Wang SJ, Wang Y, Wolfinger R, Wong A, Wu J, Xiao C, Xie Q, Xu J, Yang W, Zhang L, Zhong S, Zong Y, Slikker W Jr. The MicroArray Quality Control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements. *Nat Biotechnol* 2006; **24**: 1151-1161
20 **Rugg-Gunn PJ**, Ferguson-Smith AC, Pedersen RA. Epigenetic status of human embryonic stem cells. *Nat Genet* 2005; **37**: 585-587
21 **Sun BW**, Yang AC, Feng Y, Sun YJ, Zhu Y, Zhang Y, Jiang H, Li CL, Gao FR, Zhang ZH, Wang WC, Kong XY, Jin G, Fu SJ, Jin Y. Temporal and parental-specific expression of imprinted genes in a newly derived Chinese human embryonic stem cell line and embryoid bodies. *Hum Mol Genet* 2006; **15**: 65-75
22 **Rugg-Gunn PJ**, Ferguson-Smith AC, Pedersen RA. Status of genomic imprinting in human embryonic stem cells as revealed by a large cohort of independently derived and maintained lines. *Hum Mol Genet* 2007; **16** Spec No. 2: R243-R251
23 **Onyango P**, Jiang S, Uejima H, Shamblott MJ, Gearhart JD, Cui H, Feinberg AP. Monoallelic expression and methylation of imprinted genes in human and mouse embryonic germ cell lineages. *Proc Natl Acad Sci USA* 2002; **99**: 10599-10604
24 **Rugg-Gunn PJ**, Ferguson-Smith AC, Pedersen RA. Human embryonic stem cells as a model for studying epigenetic regulation during early development. *Cell Cycle* 2005; **4**: 1323-1326

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