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REVIEW

Potential for pharmacological manipulation of human embryonic stem cells

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The therapeutic potential of human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) is vast, allowing disease modelling, drug discovery and testing and perhaps most importantly regenerative therapies. However, problems abound; techniques for cultivating self-renewing hESCs tend to give a heterogeneous population of self-renewing and partially differentiated cells and general include animal-derived products that can be cost-prohibitive for large-scale production, and effective lineage-specific differentiation protocols also still remain relatively undefined and are inefficient at producing large amounts of cells for therapeutic use. Furthermore, the mechanisms and signalling pathways that mediate pluripotency and differentiation are still to be fully appreciated. However, over the recent years, the development/discovery of a range of effective small molecule inhibitors/activators has had a huge impact in hESC biology. Large-scale screening techniques, coupled with greater knowledge of the pathways involved, have generated pharmacological agents that can boost hESC pluripotency/self-renewal and survival and has greatly increased the efficiency of various differentiation protocols, while also aiding the delineation of several important signalling pathways. Within this review, we hope to describe the current uses of small molecule inhibitors/activators in hESC biology and their potential uses in the future.

LINKED ARTICLES
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Abbreviations
1-EBIO, 1-ethyl-2-benzimidazolinone; ADA, adenosine deaminase; ALK, activin receptor-like kinase; AMPK, AMP-activated protein kinase; AP-1, activation protein 1; ATRA, all-*trans*-retinoic acid; bFGF/FGF2, basic fibroblast growth factor; BIO, 6-bromoindirubin-3′oxime; BMP, bone morphogenetic protein; cGMP, current good manufacturing practice; CKI-7, casein kinase inhibitor; CSA, cyclosporin A; DETA-NO, diethylenetriamine NO; DMSO, dimethyl sulfoxide; DNMT, DNA methyltransferase; EB, embryoid body; EHNA, erythro-9-(2-hydroxy-3-nonyl)adenine; EPH-B3, ephrin type-B receptor 3; EpiSC, post-implantation mice embryo stem cell; FBP, flurbiprofen; FGFR, fibroblast growth factor receptor; FLT3, Fms-related tyrosine kinase 3; GGPP, geranylgeranyl pyrophosphate; GRB2, growth factor receptor-bound protein 2; GSK3β, glycogen synthase kinase 3; GTFX, gatifloxacin; HDAC, histone deacetylase; hECC, human embryonal carcinoma cell; hErbB2 (HER-2), human epidermal growth factor receptor 2; hESC, human embryonic stem cell; Hh, hedgehog; HIF, hypoxia inducible factor; hiPSC, human-induced pluripotent stem cell; HMGCoA, 3-hydroxy 3-methylglutaryl coenzyme A; Id, inhibitor of differentiation; IGF-II, insulin-like growth factor II; iPSC, induced pluripotent stem cell; K_{Ca}, calcium-activated potassium channel; KOSR, Knockout SerumTM Replacement; LIF, leukaemia inhibitory factor; LSD1, lysine-specific demethylase 1; MEF, mouse embryonic fibroblast; MEK1/MAP2K1, MAPK kinase; mESC, mouse embryonic stem cell; miRNA, micro-RNA; MNK1, MAP kinase interacting serine/threonine kinase 1; mTOR, mammalian target of rapamycin; ncRNA, non-coding RNA; NFAT, nuclear factor of activated T cells; NME2, nucleoside diphosphate kinase-B; NPC, neural progenitor cell; NSAID, non-steroidal anti-inflammatory drug; NSC, neural stem cell; PDGF, platelet derived growth factor; PDK-1, phosphoinositide-dependent kinase-1;

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PDK1, pyruvate dehydrogenase kinase 1; PDX1, pancreatic and duodenal homeobox 1; PIP2, phosphatidylinositol (3,4)-bisphosphate; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; PORCN, porcupine; PP2A, protein serine/threonine phosphatase 2A; PRK2, PKC-related kinase 2; RA, retinoic acid; ROCK, Rho-associated protein kinase; ROS, reactive oxygen species; RSK1/MSK1, ribosomal protein S6 kinase; SAHA, suberoylanilide hydroxamic acid; SHH, sonic hedgehog; SIP1, SMAD-interacting protein; SMO, smoothened; SNM, sinomenine; STAT, signal transducer and activator of transcription; THEA, theanine; TSA, trichostatin A; VPA, valproic acid

Introduction

While mouse embryonic stem cells (mESCs) use leukaemia inhibitory factor (LIF), which activates the JAK/signal transducer and activator of transcription (JAK/STAT) pathway (Niwa *et al*., 1998; Matsuda *et al*., 1999), and bone morphogenetic proteins (BMPs), which induce inhibitor of differentiation (Id) proteins via the SMAD pathway (Ying *et al*., 2003), to maintain their pluripotent nature, human embryonic stem cells (hESCs) cannot be cultivated under these conditions (Humphrey *et al*., 2004). Long-term culture of hESCs is supported by high levels of basic fibroblast growth factor (bFGF/FGF2) (Xu *et al*., 2005) and TGF-β/activin/nodal proteins (James *et al*., 2005; Vallier *et al*., 2005). The observed differences may arise due to their differing developmental origin, with hESCs representing an earlier developmental stage more akin to stem cells derived from post-implantation mice embryos (EpiSCs) (Brons *et al*., 2007; Tesar *et al*., 2007). Therefore, if the signals mediating pluripotency/self-renewal of hESCs and mESCs are dissimilar, the signals mediating differentiation of these cells may also differ. It has been noted that mESC and hESC do react differently in response to the same cellular signal, such as the addition of BMP4 to hESCs, which leads to rapid differentiation (Bernardo *et al*., 2011) while mediating self-renewal in mESCs (Qi *et al*., 2004), and FGF/ERK signalling, which promotes self-renewal in hESCs and differentiation in mESCs (Kunath *et al*., 2007). Additionally, studies suggest that hESCs exist in a state of balance and require exquisite control, with minute perturbations in the signalling pathways having huge affect, and further, that interplay between signalling pathways is vitally important.

This review will therefore attempt to bring together the current knowledge of the use of small molecule activators/inhibitors in the maintenance of the pluripotent state (summarized in Table 1) and differentiation of hESCs (summarized in Table 2).

Pharmacological control of pluripotency

Maintenance of hESC self-renewal and pluripotency

High content screens of small molecules linked to various pluripotent endpoint assays have been undertaken in an attempt to find compounds that will allow for the continued stable growth of hESCs, thereby allowing for a homogeneous

Table 1

Small molecule activators/inhibitors known to modulate the pluripotent state of hESCs

Drug	Target	Reference
Antimycin A	Mitochondrial respiratory chain	(Varum *et al*., 2009)
BIO	GSK3β	(Bone *et al*., 2009; James *et al*., 2005; Sato *et al*., 2004)
Butyryl CoA	Energy release/storage	(Ware *et al*., 2009)
CHIR99021	GSK3β	(Tsutsui *et al*., 2011)
DETA-NO	NO donor	(Tejedo *et al*., 2010)
Dorsomorphin	ALK2, 3 and 6	(Gonzalez *et al*., 2011)
EHNA	?	(Burton *et al*., 2010a,b)
FBP	?	(Desbordes *et al*., 2008)
GTFX	?	(Desbordes *et al*., 2008)
HDACs (NaB, TSA, VPA, SAHA)	Histone proteins	(Ware *et al*., 2009)
Okadaic acid	PP2A	(Yoon *et al*., 2010)
PD98059	MAP2K1/MEK1	(Armstrong *et al*., 2006; Li *et al*., 2007; Tsutsui *et al*., 2011),
SNM	Prostaglandin, leukotriene and NO synthesis	(Desbordes *et al*., 2008)
THEA	?	(Desbordes *et al*., 2008)
U0126	MAP2K1/MEK1	(Armstrong *et al*., 2006; Li *et al*., 2007)

Table 2

Small molecule activators/inhibitors known to modulate the differentiation of hESCs

Drug	Target	Pathway	Differentiated Cell Fate	Reference
1-EBIO	Ca^{2+} Activated K^{+} Channels		Cardiac and cardiac pacemaker-like cells	(Müller *et al.*, 2011)
1m	GSK3β	WNT	Primitive streak, mesoderm, definitive endoderm	(Bone *et al.*, 2011)
5-Azacytidine/5-aza-2′-deoxycytidine	DNMT's		Cardiomyocyte	(Wang *et al.*, 2010; Xu *et al.*, 2002; Yoon *et al.*, 2006)
ALK5-I/II Inhibitor + DAPT	ALK5 + γ-secretase	TGFβ + NOTCH	Pancreatic endocrine cells	(Rezania *et al.*, 2011)
ATRA	RARs/RXRs		Mainly neurogenesis	(Desbordes *et al.*, 2008)
BIO + SB431542	GSK3β + ALK4,5 & 7	WNT + TGFβ	Neural crest	(Menendez *et al.*, 2011)
BMS-189453 + NOGGIN	RARs/RXRs		Cardiomyocytes	(Zhang *et al.*, 2011)
CHIR99021 + DORSOMORPHIN + RA + SB431542	GSK3β + ALK2, 3 and 6+ ALK4,5 & 7	WNT + TGFβ	hiPSCs into definitive endoderm then pancreatic cells	(Kunisada *et al.*, 2011)
CHIR99021 + SB431542 + Compound E	GSK3β + ALK4,5 & 7 + γ-secretase	WNT + TGFβ + NOTCH	Cardiomyocytes	(Kattman *et al.*, 2011)
Chlorate	Downregulation of Sulfonation	WNT, TGFβ, and FGF/ERK	Mature neurons	(Sasaki *et al.*, 2010)
Cobalt chloride	HIF-1α stabilization		Cardiomyocyte differentiation to functionally mature cardiomyocytes	(Ng *et al.*, 2011)
Corticosteroids			Trophoblast and mesodermal	(Barbaric *et al.*, 2010)
CSA	Calcineurin	NFAT	Cardiomyocyte differentiation from ESC-derived mesodermal cells in visceral endodermal stromal cell co-culture	(Mummery *et al.*, 2003)
			Decreased hypertrophy of ESC-derived cardiomyocytes	(Lim *et al.*, 2000)
Cyclopamine	SMO	Hh	Astrocytes	(Lee *et al.*, 2006)
Cymarin	RARs/RXRs		Mesodermal/endodermal	(Desbordes *et al.*, 2008)
Dorsomorphin	ALK2, 3 and 6	TGFβ	Neurogenesis	(Kim *et al.*, 2010; Wada *et al.*, 2009; Zhou *et al.*, 2010)
Dorsomorphin + SB431542	ALK2, 3 and 6 + ALK4,5 & 7	TGFβ	Pancreatic Cells	(Nostro *et al.*, 2011)
			Neurogenesis	(Kim *et al.*, 2010; Morizane *et al.*, 2011)
IDE1 and 2		TGFβ	Definitive endoderm	(Borowiak *et al.*, 2009)
ILV + KAAD-Cyclopamine	PKCs + SMO	Hh	Pancreatic Progenitors	(Chen *et al.*, 2009; D'Amour *et al.*, 2006; Kroon *et al.*, 2008; Thatava *et al.*, 2011).
IWP-4, IWR-1		WNT	Cardiomyocytes	(Hudson *et al.*, 2011)
IWR-1		WNT	Cardiomyocytes	(Ren *et al.*, 2011)
IWR-1, IWP-3		WNT	Cardiomyocytes	(Willems *et al.*, 2011)
LY294002	PI3K	PI3K/AKT/mTOR	Endodermal	(Touboul *et al.*, 2010)

Table 2
Continued

Drug	Target	Pathway	Differentiated Cell Fate	Reference
NaB	Histone Proteins		Hepatocytes differentiation Endodermal and trophectodermal	(Hay *et al.*, 2008) (Maimets *et al.*, 2008)
Nutlin	TP53		Primitive endoderm and trophectoderm differentiation	(Maimets *et al.*, 2008)
PD98059	MAP2K1/MEK1	MEK/ERK	Haematopoietic and functional endothelial and smooth muscle cells	(Park *et al.*, 2010)
Purmorphamine	SMO	Hh	Ventral spinal progenitors and motor neurons	(Li *et al.*, 2008)
RA			Functional Insulin Producing Cells	(Jiang *et al.*, 2007)
Rapamycin	mTOR	PI3K/AKT/mTOR	Mesodermal and endodermal	(Zhou *et al.*, 2009)
			Osteogenesis	(Lee *et al.*, 2010)
Red Ginseng			Cardiac-progenitor like cells from hESC-derived EBs.	(Kim *et al.*, 2011)
Rosiglitazone	PPARγ		Adipocytic Differentiation	(Xiong *et al.*, 2005)
Sarmentogenin			Mesodermal/Endodermal	(Desbordes *et al.*, 2008)
SB203580	p38 MAPK	MAPK	Cardiomyogenesis	(Gaur *et al.*, 2010; Kempf *et al.*, 2011)
SB431542	ALK4,5 & 7	TGFβ	Primitive NPCs	(Li *et al.*, 2011)
			Myocyte progenitors	(Mahmood *et al.*, 2010)
			hESC-derived hemogenic epithelial cells into HPCs	(Wang *et al.*, 2011)
			Cardiomyocytes	(Graichen *et al.*, 2008; Xu *et al.*, 2008),
			Endothelial cells	(James *et al.*, 2010).
			hESC-derived endoderm cells into hepatic progenitors	(Touboul *et al.*, 2010)
SB431542 + CKI-7	ALK4,5 & 7 + Casein Kinase	TGFβ + LEFTYA	Retinal	(Osakada *et al.*, 2009)
SB431542 + NOGGIN	ALK4,5 & 7 + BMP	TGFβ	Neurogenesis	(Chambers *et al.*, 2009)
			Anterior Foregut Endoderm	(Green *et al.*, 2011)
			Endocrine differentiation from hESC-derived pancreatic progenitors	(Nostro *et al.*, 2011)
SB431542 + Purmorphamine	ALK4,5 & 7 + SHH	TGFβ + SHH	Motor Neuron Precursors	(Patani *et al.*, 2009)
SP60125	JNK/AP-1	Non-canonical WNT	Haematopoiesis	(Rai *et al.*, 2011)
Stauroprimide			Primes for multilineage differentiation	(Zhu *et al.*, 2009)
Synthetic RA Analogues	RARs/RXRs		Mainly Neurogenesis	(Christie *et al.*, 2008)

and plentiful source of cells for lineage-specific differentiation. Commonly used media and growth substrates are generally not well defined and may be contaminated by pathogens or xenogens (Martin *et al*., 2005). For this reason, many laboratories have attempted to develop fully defined conditions for hESC growth and in doing so have identified many cytokines and growth factors, such as WNT proteins, fibroblast growth factor (FGF), heparin, TGF-β, insulin-like growth factor II (IGF-II), activin A, platelet derived growth factor (PDGF) and neurotrophins (Dravid *et al*., 2005; Pebay *et al*., 2005; Vallier *et al*., 2005; Pyle *et al*., 2006; Xiao *et al*., 2006; Bendall *et al*., 2007; Furue *et al*., 2008; Montes *et al*., 2009) and growth surfaces (Klim *et al*., 2010; Mei *et al*., 2010; Melkoumian *et al*., 2010; Rodin *et al*., 2010; Villa-Diaz *et al*., 2010; Irwin *et al*., 2011; Lee *et al*., 2011; Nandivada *et al*., 2011; Saha *et al*., 2011), which allow for clonal feeder-free growth and subsequent differentiation. One such commercial success is the mTeSR® defined media from StemCell Technologies, which allow for both hESC and human-induced pluripotent stem cell (hiPSC) growth on Matrigel extracellular matrix with no additional growth factors (Thomson *et al*., 1998; Ludwig *et al*., 2006; Takahashi *et al*., 2007). However, the use of large amounts of highly purified growth factors and specified media for hESC growth can be very expensive, and so small molecule inhibitors/activators may be able useful for replacing these growth factors at a lower cost. To this end, a recent article has suggested that PD98059 (MAPK kinase 1, MAP2K1/MEK1 inhibitor), CHIR99021 [glycogen synthase kinase 3 (GSK3β) inhibitor] and Y27632 [Rho-associated protein kinase (ROCK) inhibitor] encompass a small molecule inhibitor cocktail that can support long-term maintenance of hESCs and allows for serial single cell passaging, following a feedback system control methodology that allowed the assay of numerous compounds at different concentrations (Tsutsui *et al*., 2011). However, it was noted that, with increases in the level of CHIR99021, differentiation occurred, demonstrating the fine balance that exists between proliferation and differentiation.

A comprehensive study from The International Stem Cell Initiative Consortium reviewed the requirements for hESC growth through a multi-laboratory comparison of the diverse methodologies utilised (Akopian *et al*., 2010). However, analysis found that of the culture systems analysed through all laboratories, only three systems supported maintenance of tested hESC lines for 10 passages; those being cultivation of cells in the presence of Knockout SerumTM Replacement (KOSR; Invitrogen) supplemented with FGF2 in the presence of a mouse embryonic fibroblast (MEFs) feeder cell layer, which was the positive control for these studies, and the two commercially available defined hESC culture media preparations: mTeSR®1 and StemPro® (Invitrogen).

Excitingly, a recent study has demonstrated the derivation and growth of hESCs that are potentially pure enough to be used in therapies and have deposited these in the UK Stem Cell banks, which will be available to laboratories across Europe (Ilic *et al*., 2011). Protocols were developed for the successful derivation of two normal and three specific mutation-carrying (Huntington's disease and myotonic dystrophy 1) genomically stable hESC lines, and their adaptation to feeder-free culture, all under completely xeno-free conditions, using human fetal fibroblast extracellular matrix as a growth substrate and TeSR™2, an improved version of mTeSR®1, as a growth medium.

WNT pathway modulation and pluripotency

The WNT signalling pathway has been shown to be vitally important to hESC self-renewal through the use of the inhibitor BIO (6-bromoindirubin-3′oxime), which is derived from the mollusc compound Tyrian purple (Meijer *et al*., 2003). BIO is a potent, reversible, ATP-competitive inhibitor of the serine-threonine kinase GSK3β, which, when inhibited activates WNT/β-catenin signalling, allowing the maintenance of the undifferentiated phenotype in both hESCs and mESCs (Sato *et al*., 2004; James *et al*., 2005). The AXIN/GSK3β/APC complex normally promotes the proteolytic degradation of β-catenin, and so if this 'β-catenin destruction complex' is inhibited, β-catenin can accumulate, stabilize and enter the nucleus and then interact with the TCF/LEF family transcription factors, which promote specific gene expression. Recent studies linking WNT signalling and pluripotency have shown that the human NANOG gene is regulated through a TCF/LEF element within an enhancer (Kim *et al*., 2011a), while a pluripotency-associated micro-RNA (miRNA) cluster (miR-371–373) (Wang *et al*., 2008; Judson *et al*., 2009) was found to be positively regulated by WNT/β-catenin signalling activity in several human cancer cell lines (Zhou *et al*., 2011). Lithium chloride (LiCl)-mediated inhibition of GSK3 and β-catenin ubiquitination (Klein and Melton, 1996) stimulated WNT/β-catenin activity and subsequently stimulated the expression of the miRNA cluster through direct binding of β-catenin/LEF1 to the miRNA promoter. Targets of the miRNAs included DKK1, a WNT/β-catenin signalling inhibitor, therefore providing a regulatory feedback loop. However, the results from one study suggest that the effect of GSK3β inhibition may be culture specific (Bone *et al*., 2009). When cultured on inactivated MEFs, BIO aided the maintenance of pluripotency; but this effect was lost upon growth on Matrigel with mTeSR® medium.

The importance of the WNT pathway in hESC pluripotency has also been shown in related studies. Firstly, treatment of hESC with okadaic acid, a potent inhibitor of protein serine/threonine phosphatase 2A (PP2A) (Garcia *et al*., 2003), promoted hESC self-renewal through the inactivation of GSK3β (Yoon *et al*., 2010). Secondly, lower oxygen levels have been shown to enhance β-catenin activity in mESCs (Mazumdar *et al*., 2010), leading to the enhancement of pluripotency. Low oxygen tension in hESC culture is known to better maintain the undifferentiated state (Ezashi *et al*., 2005; Westfall *et al*., 2008; Chen *et al*., 2010b; Lim *et al*., 2011), probably through the functions of hypoxia inducible factors (HIFs) (Forristal *et al*., 2010) but may affect the WNT signalling pathway. When mammalian cells are cultured under low oxygen tension, ATP production via oxidative phosphorylation in the mitochondria is decreased and glycolytic functions increase in order to meet energy demands. Further, antimycin A, a secondary metabolite from *Streptomyces* bacteria, has been shown to enhance hESC pluripotency through inhibition of the mitochondrial respiratory chain, which results in reduced mitochondrial oxidative phosphorylation and increased reactive oxygen species (ROS) signalling (Varum *et al*., 2009).

TGF-β pathway modulation and pluripotency

The TGF-β signalling pathway is involved in many cellular processes, including the promotion of differentiation and the TGF-β superfamily of ligands include BMPs, activin, nodal and TGF-βs. Binding of a ligand to its cell membrane receptor mediates the phosphorylation of specific SMAD proteins that can then enter the nucleus to mediate target gene expression.

Recent research has attempted to delineate the role of this complex pathway in hESC self-renewal (Xu *et al.*, 2008a; Vallier *et al.*, 2009a,b; Brown *et al.*, 2011; Mullen *et al.*, 2011). Activin/nodal signalling leads to SMAD2/3 activation, which is required to maintain hESC identity (Beattie *et al.*, 2005; James *et al.*, 2005; Vallier *et al.*, 2005; Xu *et al.*, 2008a), and SMAD3 was recently found to co-occupy OCT4 binding sites across the genome in hESCs and mESCs (Mullen *et al.*, 2011). Further analysis in mESC demonstrated that SMAD3 also co-occupied NANOG and Sox2 binding sites, and that OCT4 recruited SMAD3, although there was no evidence of a direct interaction between the two, suggesting a larger complex may be present. NANOG was also shown to be regulated through activin/nodal signalling in hESCs (Xu *et al.*, 2008a; Vallier *et al.*, 2009a) and hiPSCs (Vallier *et al.*, 2009b) through direct binding of SMAD2/3 to its promoter (Vallier *et al.*, 2009a) and also to co-operate with SMAD2/3 in hESCs to maintain pluripotency (Brown *et al.*, 2011). Additionally, SMAD2/3-NANOG inhibited ectodermal differentiation induced by FGF signalling (Xu *et al.*, 2008a; Vallier *et al.*, 2009a), again highlighting the balance required between signalling pathways for distinct outcomes. Further studies have also shown that this pathway is required for early differentiation (Brown *et al.*, 2011; Chng *et al.*, 2011; Teo *et al.*, 2011). Activin/nodal-mediated SMAD2/3 activation was observed in definitive endoderm cells, through binding of SMAD2/3 at different genomic sites to SMAD2/3-NANOG, suggesting that in endodermal differentiation SMAD2/3 interacts with another partner, such as EOMES (Teo *et al.*, 2011), changing its occupancy profile and therefore eliciting a completely different effect (Brown *et al.*, 2011). SMAD-interacting protein (SIP1) also interacts with SMAD2/3 in hESCs, and its expression is mediated by activin/nodal-regulated NANOG expression (Chng *et al.*, 2011). In hESCs, SIP1 expression limits the capacity of SMAD2/3 to differentiate towards mesendoderm, while SIP1 expression upon differentiation allows neuroectodermal differentiation mediated by activin/nodal signalling (Chng *et al.*, 2011). These new data show that signalling pathways such as these need to be studied in detail to allow the discovery of new potential targets for drug discovery.

One known compound, dorsomorphin (or compound C), was shown to promote hESC self-renewal and maintain the self-renewing, pluripotent state (Gonzalez *et al.*, 2011) through the inhibition of TGF-β/BMP type I activin receptor-like kinases (ALK2, 3 and 6) (Yu *et al.*, 2008) and thus blocking SMAD1/5/8 phosphorylation and blocking extra-embryonic differentiation, while also acting as a potent, selective, reversible, and ATP-competitive inhibitor of AMP-activated protein kinase (AMPK). This suggests that apart from boosting pluripotency, the inhibition of differentiation is also an important, and potential drug, target.

MEK/ERK and PI3K/PKB/mTOR pathway modulation and pluripotency

Both the MEK/ERK and the PI3K/PKB/mammalian target of rapamycin (mTOR) pathways have been found to be active in hESCs downstream of FGF signalling and to cooperate in enhancing pluripotency (D'Amour *et al.*, 2005; Armstrong *et al.*, 2006; Li *et al.*, 2007; McLean *et al.*, 2007). MEK/ERK signalling is required for the maintenance of hESC self-renewal as shown through the use of the MEK inhibitors PD98059 and U0126 (Armstrong *et al.*, 2006; Li *et al.*, 2007), in contrast to what is known for mESCs (Burdon *et al.*, 1999). The pathway regulates survival and proliferation in a diverse set of cells, and determines their fate(Bottcher and Niehrs, 2005), through the signal transduction of extracellular signalling mediated by cell surface receptors such as the EGF receptor, TRK A/B (common ligands of TRK receptors are neurotrophins), FGF receptor (FGFR) and PDGFR via the adaptor protein growth factor receptor-bound protein 2 (GRB2), which activates RAS/RAF, activating MEK and MAPKs, which ultimately leads to alterations in gene expression. The PI3K/PKB (PKB) pathway functions through PI3K catalysing the conversion of PIP2 [phosphatidylinositol (3,4)-bisphosphate] to PIP3 [phosphatidylinositol (3,4,5)-trisphosphate], which mediates the phosphorylation and activation of PKB through PDK-1 (phosphoinositide-dependent kinase-1) (Alessi *et al.*, 1997; Franke *et al.*, 1997), which then activates mTOR a serine/threonine protein kinase that has been shown to support self-renewal and suppress differentiation in hESCs (Zhu *et al.*, 2011).

Chromatin modulation and pluripotency

hESCs have a distinct 'open' chromatin environment associated with hyper-acetylation of histone proteins and low levels of DNA methylation, which leads to accessible DNA permissive for transcription. It is proposed that this is important for the attainment/maintenance of the pluripotent phenotype and also suggests that chemical modulation of the chromatin environment could therefore modulate pluripotency. The histone deacetylases (HDACs) sodium butyrate (NaB), trichostatin A (TSA), valproic acid (VPA) and suberoyl anilide hydroxamic acid (SAHA), which boost levels of histone acetylation, all have positive effects on hESC maintenance/self-renewal. However, NaB and its metabolite butyryl CoA, essential for immediate energy and energy storage, has the biggest affect (Ware *et al.*, 2009). Butyrate inhibits most HDACs except class III HDAC and the class IIb HDAC-6 and HDAC-10 (Davie, 2003). TSA inhibits class I and II HDACs but not class III HDACs (Sirtuins) (Vanhaecke *et al.*, 2004). VPA is an HDAC1 inhibitor, while SAHA inhibits class I and class II HDACs. Use of these inhibitors should lead to the enhancement of the open chromatin environment associated with pluripotency, and their use has also been demonstrated to promote hiPSC formation (Huangfu *et al.*, 2008; Zhu *et al.*, 2010). Other epigenetic modifications have been identified as potential therapeutic targets (Kelly *et al.*, 2010) and could have relevance to pluripotency and differentiation of hESCs. These include inhibition of lysine-specific demethylase 1 (LSD1) by parnate/tranylcypromine (Li *et al.*, 2009b), BIX-01294-mediated repression of the G9a/GLP histone lysine 9 methyltransferases (Chang *et al.*, 2009), inhi-

bition of DNA methyltransferases (DNMTs) by compounds such as 5-azacytidine/5-aza-2′-deoxycytidine and disruption/promotion of non-coding RNA (ncRNA) function, as is discussed later.

Other modifiers of pluripotency

Erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) blocks differentiation and maintains the expression of pluripotency markers in hESCs even when cultured under differentiating conditions and additionally acts as a strong blocker of directed neuronal differentiation (Burton *et al*., 2010a,b). EHNA has been found to inhibit adenosine deaminase (ADA) (Carson and Seegmiller, 1976) and the cyclic nucleotide PDE2 (Michie *et al*., 1996). However, chemically distinct inhibitors of ADA and PDE2, unlike EHNA, lack the ability to suppress hESC differentiation, suggesting that the effect of EHNA is not through the inhibition of either ADA or PDE2. Preliminary structure–activity relationship analysis found the differentiation-blocking properties of EHNA to reside in a pharmacophore comprising a close adenine mimetic. The effect of EHNA was also shown to be reversible as hESCs cultured with EHNA could faithfully differentiate to cells representative of all three germ layers after removal of the drug. Therefore EHNA or other related simple 9-alkyladenines may provide a useful replacement for bFGF in large-scale or current good manufacturing practice (cGMP)-compliant processes.

By utilising a high-throughput assay, four compounds were identified, which could promote the short-term self-renewal of hESCs; theanine (THEA), sinomenine (SNM), gatifloxacin (GTFX) and flurbiprofen (FBP) (Desbordes *et al*., 2008). THEA is a natural compound found in black tea with proposed roles in neuroprotection (Nathan *et al*., 2006) and the immune system (Kamath *et al*., 2003). Sinomenine (or cocculine) is a morphine derivative with anti-rheumatic effects thought to be primarily mediated via the release of histamine (Yamasaki, 1976); but other effects such as inhibition of prostaglandin, leukotriene and NO synthesis may also be involved (Liu *et al*., 1994). An unrelated study has shown that exposure of ESCs to low concentrations of diethylenetriamine NO (DETA-NO) adduct maintains hESC pluripotency to a similar extent as bFGF (Tejedo *et al*., 2010), although no definitive mechanism was provided. Gatifloxacin is an antibiotic of the fourth-generation fluoroquinolone family (Burka *et al*., 2005), while flurbiprofen is a member of the phenylalkanoic acid derivative family of non-steroidal anti-inflammatory drugs (NSAIDs) used to treat the inflammation and pain of arthritis. Interestingly, recent research has shown that the NSAID nabumetone can aid the reprogramming process in mouse iPSCs and can replace virally expressed c-Myc and Sox2 (Yang *et al*., 2011). Nabumetone exerts anti-inflammatory activity by inhibiting COX2 function through its metabolite 6-methoxy-2-naphthylacetic acid.

Embryonic stem cell survival

Much work has gone into finding molecules that promote the survival of hESCs, especially as cell sorting and passaging can leave cells in a single cell state, which favours apoptosis (Wong *et al*., 2004). hESCs are 'social' cells and tight junctions hold them together, offering a survival advantage over dissociated hESCs (Sathananthan *et al*., 2002). Apoptosis of dissociated hESCs has been shown to act through ROCK-dependent hyper-activation of actomyosin caused by the loss of E-cadherin-dependent intercellular contact (Ohgushi *et al*., 2010). Inhibition of ROCK, a downstream effector of Rho signalling, a master regulator of cytoskeleton remodelling and contractile force generation (Etienne-Manneville and Hall, 2002; Riento and Ridley, 2003; Li *et al*., 2010), leads to decreased phosphorylation of the myosin light chain and so inhibiting actin-myosin contractility, greatly aiding hESC survival (Chen *et al*., 2010a).

Y-27632 is selective inhibitor of p160 ROCK and promotes single cell survival and inhibits apoptosis (Watanabe *et al*., 2007; Emre *et al*., 2010). It has been found to support feeder-free hESC and hiPSC growth (Pakzad *et al*., 2010), hESC growth in 3D culture (Chayosumrit *et al*., 2010), aid cryopreservation (Martin-Ibanez *et al*., 2008; Baharvand *et al*., 2010) and is now widely used in hESC growth and manipulation. More survival compounds of greater specificity, equivalent potency and reduced toxicity relative to Y-27632 were discovered in another study (Andrews *et al*., 2010). All pro-survival compounds (18 confirmed hits with four structural classes being represented by multiple compounds) were found to target ROCK/PKC-related kinase 2 (PRK2) kinases *in vitro*, which are thought to act in concert in cytoskeletal signalling (Darenfed *et al*., 2007). An exception is the K^+-ATP channel opener pinacidil (Grover, 1997), which may promote survival by 'off-target' inhibition of ROCK/PRK2 (Andrews *et al*., 2010). Two of the compounds discovered inhibited the receptor tyrosine kinase ephrin type-B receptor 3 (EPHB3) known to be involved in cell–cell signalling (Pasquale, 2005). Two other compounds identified are structurally related to tyrosine kinase inhibitors known to have effects on hESC differentiation (Anneren *et al*., 2004; Vallier *et al*., 2005) and in this study promoted mesodermal differentiation (Andrews *et al*., 2010).

Thiazovivin, a 2,4-disubstituted thiazole, and tyrintegin, a 2,4-disubstituted pyrimidine, were found to increase survival of disassociated hESCs by enhancing integrin signalling (Xu *et al*., 2010). Thiazovivin was also found to inhibit ROCK activity and protect hESCs in a manner akin to Y-27632 (Xu *et al*., 2010). Another ROCK inhibitor, HA/HA1077, was found to increase hESC survival alongside several small molecule inhibitors of PKC, which may modulate hESCs survival similar to PKC-mediated control of pluripotency in mESCs (Heo and Han, 2006). In the same study several pathways were identified that upon inhibition by specific inhibitors lead to decreased hESC survival; tyrophostin AG-1478 (EGF receptor signalling), SP600125 (JNK signalling), AG-879 [TrkA or human epidermal growth factor receptor 2 (hErbB2/neu) signalling], tyrphostin 9 (PDGF signalling) and Bay11-7082 (NF-κB) signalling), suggesting the importance of these signalling pathways to hESC self-renewal. Another study confirmed that Y-27632, HA1004, HA1077, H-89 (all kinase inhibitors) and pinacidil promote hESC viability, (Barbaric *et al*., 2010b), overall suggesting that the activities of multiple kinases, such as PRK2, ROCK, MAP kinase interacting serine/threonine kinase 1 (MNK1) and ribosomal protein S6 kinases (RSK1 and MSK1), may all be necessary for the survival of hESCs. A recent report has additionally shown that pinacidil and Y-27632 aid cryopreservation of hESCs (Barbaric *et al*.,

2011). Finally, Y-27632 has also proven to be important during hESC differentiation. It probably acts to allow increased survival of hESC-derived progeny, such as cardiomyocytes (Braam *et al*., 2010), but it has been observed to directly enhance differentiation of hESC towards neural-crest like cells (Hotta *et al*., 2009). However, it has also been shown to have a detrimental effect on haematopoietic differentiation of hESCs (Yung *et al*., 2011).

Factors inducing pluripotency

The generation of hESC-like cells from somatic cells through the forced expression of important pluripotency-associated transcription factors such as OCT4, SOX2, KLF4, C-MYC (Takahashi *et al*., 2007) or OCT4, SOX2, NANOG and LIN28 (Yu *et al*., 2007) has invigorated the field of embryonic stem cell research. iPSC technology promises to give us a source of patient-specific pluripotent cells, which can be used for cell replacement therapy through directed differentiation and also allow disease modelling and patient-specific and disease-specific drug testing. Work in hiPSCs has also uncovered a number of small molecule modulators of important signalling pathways that can promote reprogramming to the pluripotent state or take the place of pluripotency-associated transcription factors, such as C-MYC or KLF4, by acting alone or in conjunction with other inhibitors.

These include small molecules which modulate important pathways such as the WNT, TGF-β, MEK and FGF pathways, such as GSK3β (CHIR99021, LiCl) (Ying *et al*., 2008; Li *et al*., 2009a,b; Yu *et al*., 2011; Wang *et al*., 2011b), MEK (PD0325901) (Ying *et al*., 2008; Lin *et al*., 2009; Li *et al*., 2009a; Zhu *et al*., 2010; Yu *et al*., 2011), FGFR (SU5402, PD173074) (Ying *et al*., 2008), TGF-β1 ALKs (SB431542, A83-01) (Li *et al*., 2009a; Lin *et al*., 2009; Yu *et al*., 2011), the lysine specific demethylase LSD1 (parnate/tranylcypromine) (Li *et al*., 2009b) and HDACs (NaB, VPA, TSA) (Huangfu *et al*., 2008; Mali *et al*., 2010; Zhu *et al*., 2010). Other small molecule compounds promote survival (thiazovivin) (Lin *et al*., 2009), dampen the senescence response during reprogramming (vitamin C) (Esteban *et al*., 2010) or activate pyruvate dehydrogenase kinase 1 (PDK1), facilitating a conversion from mitochondrial oxidation to glycolysis (PS48) (Zhu *et al*., 2010). The functions of such inhibitors in the attainment of pluripotency should allow us to further understand the biological pathways that determine the pluripotent nature of these cells and the ability for multilineage development.

The differences between human and mouse biology may even affect the effect of reprogramming drugs. At least one report has suggested that different kinase inhibitors affect mouse and human reprogramming differently (Hirano *et al*., 2011). Mouse iPSCs cultured with MEK (PD0325901) and GSK3β (CHIR99021) inhibitors plus LIF results in the enrichment of germ-line competent ESCs, whereas hiPSCs cultured under the same conditions form bowl-shaped multi-potent stem cells with gene expression profiles resembling primitive neural stem cells (NSCs). Although, again, this difference in requirements of factors for the attainment of pluripotency is likely to be due to differences in the developmental time at which hESC and mESC are derived.

Selecting cell populations

There are several problems with the culture and differentiation of hESCs for subsequent clinical use, other than the previously mentioned problems with animal products in culture media. These include the presence of partially differentiated hESCs, which may respond in a different manner to differentiation signals compared to fully pluripotent hESCs. This increases the possibility of abnormal hESCs growth and the persistence of pluripotent cells after differentiation and upon transplantation with potentially tumourigenic risk. Therefore, the ability to control these problems would increase differentiation efficacy and reduce the risk of tumourigenesis.

High levels of statin drugs can selectively inhibit the growth of karyotypically abnormal hESCs and cancer cells eventually leading to cell death (Gauthaman *et al*., 2007; 2009). Statins are 3-hydroxy 3-methylglutaryl coenzyme A (HMGCoA) reductase inhibitors, which prevent the conversion of HMG-CoA to mevalonate and the subsequent production of downstream products, such as the isoprenoid precursor geranylgeranyl pyrophosphate (GGPP). An inhibitor of the GGPP transferase (GGTI-298) had the same effect as the statins on abnormal hESCs and cancer cells, suggesting that geranylgeranylation is the main mechanism behind abnormal cell inhibition. The development of drugs such as these may be very important in the light of recent work showing widespread genetic abnormalities in ESCs and iPSCs in culture and also abnormalities that arise during the reprogramming process for the attainment of iPSCs (Gore *et al*., 2011; Hussein *et al*., 2011; Laurent *et al*., 2011; Lister *et al*., 2011; Martins-Taylor *et al*., 2011; Taapken *et al*., 2011; Ji *et al*., 2012).

Cells on the periphery of hESC colonies generally show some spontaneous differentiation with markers of neuronal differentiation evident (Ginis *et al*., 2004; Ward *et al*., 2006), with neuronal differentiation being the default differentiation pathway in a large number of hESC lines (Smukler *et al*., 2006). Ceramide, a bioactive sphingloid, has been found to selectively target and eliminate cells expressing neuronal markers, leaving undifferentiated hESCs unaffected in long term cultures (Salli *et al*., 2009). Ceramide itself is an endogenous molecule biosynthesized and metabolized by hESCs (Brimble *et al*., 2007) and so is an attractive target for use in long-term stable hESC cultures.

A further study identified factors to which hiPSCs were more sensitive to than fibroblasts and therefore could be used as possible anti-teratogenic agents for stem cell therapy by removing unwanted iPSCs from a differentiated culture (Conesa *et al*., 2011). Benzethonium chloride and methylbenzethonium chloride, both analogue quaternary ammonium salts used as broad-spectrum antimicrobial agents, reduced iPSC viability at a lower concentration compared with two fibroblasts cultures. By similar means, it was found that the anti-arrhythmic agent amiodarone was selectively toxic to hESC-derived NSCs but not to differentiated neurons or glial cells (Han *et al*., 2009), allowing the depletion of unwanted contaminating precursor cells from a differentiated cell product in a heterogeneous culture. Amiodarone is also known to have some thyroid hormone-like activity, and binding to the nuclear thyroid receptor might contribute

to some of its pharmacological actions (Matsubara *et al.*, 2011).

A further interesting study showed the capability of a compound to elicit its effect on hESCs after transplantation (Hara *et al.*, 2010). This study demonstrated that transplantation of hESCs into the mouse retina caused immature teratoma growth with the destruction of the retinal structure. However, if mice were treated with methotrexate, a folate antagonist, at the time of hESC transplantation, the vast majority of the cells demonstrated neural differentiation in the retina (Hara *et al.*, 2010). This suggests that post hESC transplantation treatment with small molecule compounds could aid differentiation, integration and reduce teratogenic risk.

Pharmacological control of differentiation

Studies published in 2011 alone have demonstrated the huge potential of ESC and iPSC-derived cells, through the implementation of efficient differentiation protocols, to alleviate symptoms in mouse models of human disease. Such diseases/disorders include Parkinson's disease (Chung *et al.*, 2011; Kriks *et al.*, 2011; Kim *et al.*, 2011b), retinal degeneration (Tucker *et al.*, 2011), spinal chord injury (Nori *et al.*, 2011), hypopigmentation disorders (Nissan *et al.*, 2011), Alzheimer's disease (Bissonnette *et al.*, 2011) and orthopaedic disease (Bilousova *et al.*, 2011), and efficient protocols for derivation of specific cell types from hESC and hiPSCs may lead to the use of such cells to treat human disease. To this end, multiple small molecule drugs that can modulate the differentiation of clinical-grade hESCs (Ilic *et al.*, 2011) or hiPSCs have been discovered and may be used in the future in cGMP-compliant differentiation protocols to produce transplantable cells. Refinements in differentiation protocols, such as the application of such drugs, reducing cell time in culture and starting with a good source of hESCs, may all contribute to providing a source of karyotypically and phenotypically stable cells for transplantation purposes.

As expected, modulation of signalling pathways important to pluripotency leads to the differentiation of ESC down multiple lineages. In some cases, simply the removal of one factor will allow differentiation (for example bFGF), but treatment with specific inhibitors/activators also allows us to 'push' cells down certain lineages. In many cases, pathways involved in the maintenance of pluripotency prove also to be important in differentiation and so suggest that many factors may have a dose-dependent effect; and further, their roles may be affected by the stimulation/inhibition of other pathways (Vallier *et al.*, 2009b,c).

WNT pathway-mediated hESC differentiation

Modulation of WNT signalling through GSK3β inhibition has been shown to influence the differentiation of hESCs, mainly by enhancing mesodermal and cardiac differentiation. One report suggested that compound 1 m, a potent inhibitor of GSK3β identified in a large-scale screen of compounds, can maintain mESC self-renewal (Bone *et al.*, 2009) and promote differentiation towards primitive streak, mesoderm and definitive endoderm through elevated NODAL signalling (Bone *et al.*, 2011). Another large-scale screening assay identified a small molecule that inhibited transduction of the canonical WNT response leading to the potent generation of cardiomyocytes from hESC-derived mesoderm cells (Willems *et al.*, 2011). Notably, several other WNT inhibitors are very efficient at inducing cardiogenesis. including a molecule that prevents WNTs from being secreted by the cell (Willems *et al.*, 2011). hESCs adapted to single cell passaging in a 2D culture format that were induced towards cells of the primitive streak, by using BMP4 and activin A, were potently differentiated towards a cardiogenic fate through the inhibition of WNT signalling using the small molecules IWP-4 and IWR-1 (Hudson *et al.*, 2011). IWP-4 and IWR-1 act by inhibiting the palmitylation of WNT proteins by porcupine (PORCN), a membrane-bound O-acyltransferase, thereby blocking WNT secretion and activity (Chen *et al.*, 2009a). An additional study demonstrated that following BMP4-treatment of hESCs and hiPSCs, IWR-1 significantly improved cardiomyocyte differentiation resulting in cells with typical electrophysiological functions and pharmacological responsiveness (Ren *et al.*, 2011). An interesting recent study demonstrated that successive, mutually exclusive waves of non-canonical and canonical WNT signalling precede mesoderm differentiation, and blocking these two waves leads to differential differentiation (Rai *et al.*, 2011). Blocking initial non-canonical JNK/activation protein 1 (AP-1) signalling with SP60125 promotes haematopoiesis, whereas blocking the subsequent canonical WNT signalling using DKK1 promotes cardiovascular differentiation (Rai *et al.*, 2011).

Besides its importance in cardiac differentiation, BIO-mediated antagonism of WNT signalling, in combination with inhibition of SMAD signalling with SB431542 (discussed in the next section), can also mediate the specification of neural crest cells, partly through diverting differentiation from an neural progenitor cell (NPC) fate (Menendez *et al.*, 2011).

TGF-β pathway-mediated hESC differentiation

As mentioned before, SB431542 is a TGF-β1 ALK inhibitor, which is selective and potent for ALK4/5/7 while not affecting more divergent BMP signalling utilizing ALK1/2/3/6 (Inman *et al.*, 2002; Laping *et al.*, 2002) and has been shown to aid the attainment of pluripotency in hiPSCs when used in conjunction with PD0325901, an inhibitor of the MAPK/ERK pathway (Lin *et al.*, 2009). However, it has also been shown to participate in the differentiation of hESCs down various lineages.

SB431542 treatment of hESC increased neuroectoderm specification in hESC-derived embryoid bodies (EBs) (Smith *et al.*, 2008); while, similarly, treatment of hESCs with SB431542 for 8 days in non-adherent culture conditions led to the efficient and accelerated neural conversion of hESCs with negligible mesendodermal, epidermal or trophectodermal contribution (Patani *et al.*, 2009). The same group went on to show that further treatment with FGF2, retinoic acid (RA) and the sonic hedgehog (SHH) agonist purmorphamine led to the specification of motor neuron precursors (Patani *et al.*, 2011). Dual inhibition of SMAD signalling by SB431542 and NOGGIN (a natural BMP antagonist) in undifferentiated

hESCs on Matrigel-coated dishes in conditioned medium supplemented with the ROCK inhibitor Y-2763 and ascorbic acid (vitamin C) led to the rapid and complete neural conversion of around 80% of hESC (Chambers *et al*., 2009), bypassing the necessity for EB formation. Dual inhibition appears to promote efficient differentiation through the inhibition of self-renewal and the inhibition of certain lineage-specific differentiation pathways (trophectodermal, mesodermal and endodermal), thereby 'pushing' the cell down another lineage-specific pathway (ectodermal–neuronal).

Dorsomorphin was found to promote hESC maintenance and self-renewal through SMAD inhibition (Yu *et al*., 2008; Gonzalez *et al*., 2011) but can also mediate neural differentiation at the expense of mesoderm and endoderm differentiation (Kim *et al*., 2010). Again, dual inhibition of SMAD signalling through dorsomorphin and SB431542 treatment efficiently allowed several hESC and hiPSC lines to differentiate towards the neural lineage (Kim *et al*., 2010; Morizane *et al*., 2011). However, one study has demonstrated that neural conversion of hESCs and hiPSCs was maximal, with dorsomorphin alone giving a differentiation rate of 88.7% and 70.4%, respectively, and the addition of SB431542 did not increase the differentiation (Zhou *et al*., 2010). Of further interest was their finding that dorsomorphin was ineffective at inducing neural conversion in mESCs, demonstrating that small molecules may have species-specific effects (Zhou *et al*., 2010). Additionally, it was demonstrated that dorsomorphin is important in the initial differentiation of NSCs/NPCs for the induction of spinal motor neuron differentiation from hESCs (Wada *et al*., 2009).

Combined treatment of hESCs with human LIF (hLIF), CHIR99021 (GSK3β inhibitor) and SB431542, leads to the production of a cell population with features of primitive neuroepithelium (Li *et al*., 2011). Addition of a further small molecule inhibitor of γ-secretase (compound E) (Seiffert *et al*., 2000) led to the production of a primitive NSC population with remarkably high neurogenic propensity, broad differentiation potential, responsiveness to extrinsic morphogens for subsequent development into subtype-specific neuronal identities and the ability to integrate *in vivo* (Li *et al*., 2011). Overall, dorsomorphin and SB431542 seem to mediate neural differentiation and may act by potentiating the neural differentiation pathway that seems innate in differentiating hESCs.

However, SB431542 has shown some efficacy at promoting differentiation towards other lineages. SB431542 treatment of hESC-derived EBs in serum-free medium markedly up-regulated paraxial mesodermal markers and led to the production of myocyte progenitor cells, which could be further differentiated to mesenchymal progenitors that subsequently develop into osteoblast, chondrocyte and adipocyte lineages both *in vitro* and *in vivo* (Mahmood *et al*., 2010). SB431542 also promoted the transition of hESC-derived hemogenic epithelial cells into $CD43^+$ hematopoietic progenitor cells (HPCs) (Wang *et al*., 2011a) as well the retinal differentiation of hESC and hiPSCs in a serum- and feeder-free floating aggregate culture when combined with a casein kinase inhibitor (CKI-7), to mimic LEFTYA, and Y-27632 (Osakada *et al*., 2009).

Furthermore, SB431542 has been shown to aid cardiomyocyte differentiation from hESCs (Graichen *et al*., 2008; Xu *et al*., 2008b), and in the production of endothelial cells through an ID1-dependent mechanism (James *et al*., 2010). Cardiomyocyte differentiation from hESCs and hiPSCs is also boosted by the combination of dorsomorphin and SB431542, which inhibit SMAD signalling (Kattman *et al*., 2011). SB431542 promoted the differentiation of hESC-derived endoderm cells into hepatic progenitors (Touboul *et al*., 2010). This effect of SB431542 was also observed in a study where it was further demonstrated that LY294002-mediated repression of PI3K (Vlahos *et al*., 1994) allowed for increased endoderm differentiation. LY294002 is a morpholine derivative of quercetin (Maira *et al*., 2009) and has been shown to be required for the actions of activin A in specifying definitive endoderm (McLean *et al*., 2007). Dual treatment of hESCs with SB431542 alongside BMP inhibition by NOGGIN has also been shown to allow for the generation of anterior foregut endoderm from hESCs and hiPSCs (Green *et al*., 2011) and endocrine differentiation from hESC-derived pancreatic progenitors (Nostro *et al*., 2011), while also demonstrating a role of dorsomorphin in pancreatic differentiation from hESCs. Additionally, the pancreatic endocrine phenotype can also be promoted by inhibition of the TGF-β signalling pathway through either ALK5 inhibitor I or ALK5 inhibitor II combined with a γ-secretase inhibitor, which indirectly inhibits Notch (DAPT) (Rezania *et al*., 2011), and also through combined treatment with activin A and CHIR99021 to induce efficient differentiation of hiPSCs into definitive endoderm and then dorsomorphin, RA and SB431542 to efficiently induce pancreatic differentiation (Kunisada *et al*., 2011).

Lastly, it has found that the ability of a compound to boost the TGF-β pathway could aid specific differentiation (Borowiak *et al*., 2009). In a study assaying for factors that can increase endoderm differentiation from hESCs, two structurally similar small molecules, IDE1 and 2, products of *de novo* chemical synthesis identified from a library of putative HDAC inhibitors, were found to induce definitive endoderm from hESCs, in part via activation of TGF-β signalling, and were more effective at doing this than either activin A or NODAL, commonly used protein inducers of endoderm (Borowiak *et al*., 2009). The involvement of the TGF-β signalling pathway in this effect was shown through the elevation of SMAD2 phosphorylation; however, the specific biochemical targets of these small molecules are not known.

MEK/ERK and PI3K/PKB/mTOR pathway modulation and differentiation

Modulation of the MEK/ERK signalling pathway through inhibition of MEK1/2 with PD98059 alongside the presence of BMP4 has been shown to be efficient at generating $CD34^+$progenitor cells from both hESCs and hiPSCs (Park *et al*., 2010). Further differentiation of these cells allowed the production of functional endothelial and smooth muscle cells, as demonstrated by their contribution to neovasculogenesis in a mouse model of ischaemic hind limb injury. The potential for successful applications such as this have led to a great deal of interest in the differentiation of endothelial/vascular cells from hESCs (Kane *et al*., 2010; 2011) for therapeutic use. VEGFs, PDGFs, ROS and TGF-β, WNT and NOTCH signalling, alongside histone modifications and miRNAs, have all been shown to play important roles in the differentiation of endothelial and vascular smooth muscle cells pro-

viding possible druggable targets (Kane *et al.*, 2011), and providing the information required to delineate feeder-free and serum-free protocols for efficient differentiation (Kane *et al.*, 2010).

Rapamycin, a bacterial macrolide and a highly specific inhibitor of mTOR, was found to enhance mesodermal and endodermal differentiation, impair pluripotency and prevent cell proliferation of hESCs (Zhou *et al.*, 2009) and, in another study, to be a potent activator of osteogenic differentiation, concomitant with its ability to increase SMAD1/5/8 phosphorylation and Id1–4 mRNA expression (Lee *et al.*, 2010). After the induction of both hESCs and EBs for 2–3 weeks with rapamycin, osteoblastic differentiation was observed, including alizarin red S staining for mineralized bone nodule formation (Lee *et al.*, 2010).

Chromatin landscape modulation in hESC differentiation

As expected, modulation of the chromatin environment plays a role in hESC differentiation, probably by increasing the access to lineage specific gene promoters to factors induced upon the induction of differentiation. NaB can be used to promote endodermal differentiation by activin A, allowing subsequent treatment with DMSO to induce hepatocyte differentiation (Hay *et al.*, 2008). NaB has also been shown to promote the rapid differentiation of hESCs to primitive endoderm and trophectoderm lineages induced by nutlin, a small molecule activator of p53 (Maimets *et al.*, 2008). Cardiomyocyte differentiation has been demonstrated to be enhanced by 5-azacytidine/5-aza-2′-deoxycytidine (Xu *et al.*, 2002; Yoon *et al.*, 2006; Wang *et al.*, 2010), a chemical analogue of cytidine that acts as a false substrate for DNA methyltransferases, therefore reducing cellular DNA methylation content. A reduction in DNA methylation, similar to an increase in histone acetylation, induces the reactivation of genes associated with the differentiation of hESCs, and thereby primes them for appropriate signals to allow lineage-specific differentiation.

MAPK pathway-mediated hESC differentiation

Inhibition of the MAPK pathway is involved in cardiomyogenesis, demonstrated through the use of the p38 MAPK inhibitor SB203580 (Gaur *et al.*, 2010; Kempf *et al.*, 2011). Addition of this inhibitor increased the number of spontaneously beating human EBs 2.1-fold after 21 days of differentiation (Gaur *et al.*, 2010). It has also been demonstrated that treatment of hESC-derived EBs with 5 μM SB203580 increased cardiomyogenesis, but at higher concentrations of SB203580 this effect was completely absent (Kempf *et al.*, 2011). This again suggests that tight control over signalling pathways is required for hESC manipulation. Low doses of nicotine have also been found to improve the survival of transplanted hESC-derived endothelial cells, and enhance their angiogenic effects *in vivo*, through MAPK and PKB signalling pathways (Yu *et al.*, 2009).

The effects of electrical field stimulation on ROS generation and cardiogenesis in EBs derived from hESCs have also been explored and, under optimal conditions, cardiac differentiation induced by EFS was observed to be similar to that after H_2O_2 treatment (Serena *et al.*, 2009). Further the growth of hESCs in ROS-inducing conditions (BSO treatment, which inhibits intracellular glutathione and enriches ROS levels) has been shown to induce an up-regulation in mesodermal and endodermal differentiation and this occurred through MAPK signalling (Ji *et al.*, 2010). These studies are the first to demonstrate ROS-mediated differentiation in hESCs.

Retinoid-mediated hESC differentiation

RA and All-*trans*-RA (ATRA, vitamin A) are well known for their ability to boost neuronal differentiation from pluripotent stem cells (Duester, 2008). However, RA and ATRA are readily degraded in culture, reducing their long-term usefulness. This problem was addressed in a study utilising human embryonal carcinoma cells (hECCs) and it was demonstrated that synthetic analogues of RA can be more stable and effective, while some related analogues can actually mediate differentiation towards another lineage (Christie *et al.*, 2008). This suggests that structure–activity relationship information for many compounds could further our ability to design more targeted compounds capable of mediating robust and reproducible tissue differentiation.

Apart from neuronal differentiation, RA treatment of hESCs, combined with activin A aids subsequent differentiation of functional insulin-producing cells (Jiang *et al.*, 2007). ATRA has also been identified in a high-throughput screening of differentiating-inducing compounds, which also found several potent inhibitors of self-renewal and promoters of differentiation (Desbordes *et al.*, 2008). Other compounds discovered to promote mesendodermal and endodermal differentiation include cymarin, a cardiac glycoside used to treat a variety of tumours, and sarmentogenin, which is closely related to digitoxigenin. Interestingly, the pan-RA receptor antagonist BMS-189453 can significantly increase the cardiac differentiation efficiency of hESCs when used in combination with NOGGIN (Zhang *et al.*, 2011).

Hedgehog-mediated hESC differentiation

The hedgehog (Hh) pathway plays a key role in a wide variety of developmental processes in the developing embryo (Ingham and McMahon, 2001). High-content screening using a chemical library of 5000 compounds to identify small molecules that can increase the number of pancreatic and duodenal homeobox 1 (PDX1)-expressing cells derived from hESCs found one molecule, ILV, which inhibits PKC isozymes (Irie *et al.*, 2002) that when combined with growth factors, including KAAD-cyclopamine (Chen *et al.*, 2002), directed the differentiation of hESCs such that greater than 45% of the cells become PDX1-expressing pancreatic progenitors (Chen *et al.*, 2009b). KAAD-cyclopamine, a steroid alkaloid isolated from the corn lily (*Veratrum californicum*), has been identified as a specific inhibitor of Hh signalling through direct binding to the heptahelical bundle of smoothened (SMO), and ILV have been further linked to enhanced pancreatic endoderm differentiation in numerous other studies (D'Amour *et al.*, 2006; Kroon *et al.*, 2008; Thatava *et al.*, 2011). SMO is a GPCR protein in the Hh pathway, which can activate the GLI transcription factors that determine the fate of a cell fate (Ruiz i Altaba, 1999). Inhibition of the SMO pathway could allow for a more potent effect of ILV in pancreatic differentiation.

Interestingly, cyclopamine treatment of hESC followed by culture in specific astrocyte medium induced the production of cells of the astrocytic lineage (Lee *et al*., 2006), suggesting that attenuation of the Hh signalling promotes multi-lineage differentiation.

Purmorphamine is a small molecule agonist of the SMO pathway (Sinha and Chen, 2006) that has been shown to promote the specification of motor neuron precursors (Patani *et al*., 2011). Further, it has also been shown to promote the differentiation of ventral spinal progenitors and motor neurons from hESCs in the place of SHH (Li *et al*., 2008), thereby demonstrating that specific up-regulation and down-regulation of the Hh pathway can influence hESC differentiation.

Further regulators of hESC differentiation

Treatment of hiPSCs-derived EBs with 1-EBIO (1-ethyl-2-benzimidazolinone) for 10 days was found to be sufficient to mediate differentiation towards cardiac and cardiac pacemaker-like cells (Müller *et al*., 2011). 1-EBIO increases the activity of calcium-activated potassium channels (K_{Ca}s), which exhibit small (K_{Ca}2.1-2.3) or intermediate (K_{Ca}3.1) unitary conductance for K^+ ions. A previously mentioned compound, pinacidil, was found to aid the survival of hESCs (Andrews *et al*., 2010; Barbaric *et al*., 2010a,b), suggesting that such ion channel control may be very important for regulating hESC pluripotency and differentiation.

In a screen searching for factors able to boost endoderm differentiation, the compound stauroprimide was found to 'prime' hESCs for differentiation towards multiple lineages using appropriate lineage-specifying conditions following treatment (Zhu *et al*., 2009). Stauprimide is structurally similar to the natural product staurosporine, and the staurosporine analogue UCN-01, which are widely used as non-specific kinase inhibitors (Ruegg and Burgess, 1989). However, stauprimide did not have any obvious effects on most kinases tested, except for Fms-related tyrosine kinase 3 (FLT3) and MLK1. Further analysis found that stauroprimide targets nucleoside diphosphate kinase-B (NME2) (Zhu *et al*., 2009); and by binding to NME2, stauprimide inhibits NME2 nuclear localization (Zhu *et al*., 2009), which, in turn, represses C-MYC expression (Thakur *et al*., 2009). This suggests that the attenuation of a pluripotency associated transcription factor may allow for the initiation of multi-lineage differentiation.

Cyclosporin A (CSA) treatment of hiPSCs at the mesoderm differentiation stage in visceral endodermal stromal cell co-culture-mediated cardiomyocyte differentiation (Mummery *et al*., 2003) led to an increased number of beating colonies, although direct treatment of the undifferentiated hiPSCs themselves yielded no effect (Fujiwara *et al*., 2011). CSA is an immunosuppressant and a calcineurin inhibitor that is thought to function through the inhibition of nuclear factor of activated T cells (NFAT) signalling in T cells (Crabtree and Olson, 2002). It has also been shown to have some effects on cardiac myocytes through decreased hypertrophy (Lim *et al*., 2000). CSA-treated human iPSC-derived cardiomyocytes have the same various cardiac marker expressions, synchronized Ca^{2+} transients, cardiomyocyte-like action potentials, pharmacological reactions and ultrastructural features as usual cardiomyocytes (Fujiwara *et al*., 2011). Treatment of hESCs with cobalt chloride boosts the differentiation of cardiomyocytes to functionally mature cardiomyocytes by inducing the stabilization of HIF-1α (Ng *et al*., 2011), thereby chemically mimicking a reduction in oxygen concentration.

A previously mentioned study assaying for compounds that enhance hESC-survival also identified corticosteroid drugs as being potent enhancers of differentiation (Barbaric *et al*., 2010b). Corticosteroids normally exert their effect by binding to steroid hormone receptors (Lowenberg *et al*., 2008); prednisolone, 6-α-methylprednisolone, betamethasone and dexamethasone were all found to reduce OCT4 expression in hESCs and increase markers of the trophoblast and mesodermal lineages, suggesting that these compounds could be useful tools for lineage priming of hESCs (Barbaric *et al*., 2010b).

A study into adipocyte differentiation from hESCs found that treatment with rosiglitazone, a PPARγ agonist and anti-diabetic drug in the thiazolidinedione class, enhanced the percentage of adipocytes that differentiated and the adipocyte-specific hormone leptin (Xiong *et al*., 2005), in line with a suggested master regulator role for PPARγ in adipogenesis (Rosen and Spiegelman, 2000). This establishes a method for directing adipocyte differentiation from hESCs.

Red ginseng (*Panax ginseng*) extract has also been shown to increase the proliferation of undifferentiated hESCs and enhance the expression of pluripotency-associated markers (Kim *et al*., 2011d). However, when it was added during EB-mediated differentiation, mesendoderm markers were elevated and after further culture it promoted differentiation into early stage cardiac progenitor-like cells. Falcarinol, a 17-carbon diyne fatty alcohol isolated from red ginseng, may have potent anticancer properties (Kobaek-Larsen *et al*., 2005); while other acetylenic fatty alcohols in ginseng (panaxacol, panaxydol and panaxytriol) have antibiotic properties.

Chemical down-regulation of sulfation with chlorate has been found to enhance the neural differentiation of hiPSCs (Sasaki *et al*., 2010), possibly by reducing the sulfation of several sulfur-containing proteins, such as glycoproteins, glycolipids and proteoglycans. Differentiation into mature neurons was upregulated markedly in chlorate-treated EBs, and work established in mESCs shows that this is possibly due to reduced levels of heparin sulfate and chondroitin sulfate causing defects in WNT/β-catenin, BMP/SMAD and FGF/ERK signalling (Sasaki *et al*., 2009).

Future targets

Although the benefits of pharmacological manipulation of human pluripotent stem cells are apparent, there are potential drawbacks/limitations. The long-term effects of compounds must be investigated, as well as potential for non-specific actions. Additional in depth studies of embryonic development are also required in order that biology can guide drug discovery, allowing us to understand when we use a compound, the specific amount of a compound required and the duration of exposure. Furthermore, the cost of drug discovery and development may also become prohibitive for multiple pathways and multiple targets. However, future

studies should provide more targets for pharmacological intervention.

Compound discovery and evolution

The small molecules that have been discovered have often been found through breakthroughs in the understanding of the basic biology of hESCs, and so each new level of understanding of the pluripotent state and multi-lineage differentiation brings us more potentially druggable targets. Therefore, further basic research coupled with large-scale drug screens, with appropriate read-outs, should allow for the discovery of new, more effective, defined and cost-effective compounds. As has been shown for RA (Christie *et al.*, 2008), it may also be possible to evolve compounds creating synthetic analogues of known regulators and this may be an efficient means of discovering more effective compounds.

Targeting non-coding RNA

Most druggable targets in hESCs are proteins, but RNA can also adopt complex secondary structures capable of specific ligand binding (Thomas and Hergenrother, 2008) and therefore may be an attractive target for small molecule intervention. ncRNA function has come to be understood as being a vitally important level of control in hESC self-renewal/ pluripotency and during differentiation. Therefore, the targeting of ncRNA molecules such as long non-coding RNAs (lncRNAs) (Guttman *et al.*, 2011) and miRNAs (Tiscornia and Izpisua Belmonte, 2010; Yi and Fuchs, 2011) by specific small molecule inhibitors or activators could hold much promise (Watashi *et al.*, 2010; Georgianna and Young, 2011).

Metabolomics

Recent studies have begun to characterize the metabolome of ESCs with the target of finding specific endogenously occurring small molecules that are the products of biochemical reactions, revealing connections between different pathways. This is the reverse mechanism to current drug discovery, and could lead to the discovery of more specific, more effective and importantly less toxic inhibitors/activators of certain pathways. An early proof of concept study (Cezar *et al.*, 2007), investigated the metabolome of hESCs following treatment with the HDAC inhibitor VPA and found an up-regulation in kynurenine, which controls 5-HT levels through tryptophan availability, glutamate, hydroxyproline and candidate metabolites of GABA.

One untargeted metabolomics assay has found a unique metabolic signature in mESCs characterized by metabolites that are reactive to oxygenation and hydrogenation, making them chemically useful (Yanes *et al.*, 2010). This study found a link between the eicosanoid signalling pathway and pluripotency and several oxidized metabolites and the promotion of neuronal and cardiac differentiation. A previously mentioned study found that an increase in ROS, which would lead to an increase in oxidized metabolites, led to cardiac differentiation (Serena *et al.*, 2009) and mesodermal/ endodermal differentiation (Ji *et al.*, 2010). Oxygen tension may also affect differentiation (Chen *et al.*, 2010b; Lim *et al.*, 2011) as, similar to hESC culture, differentiation protocols do no tend to use physiological levels of oxygen, as is shown in the production of retinal progenitor cells (Bae *et al.*, 2011), mesoderm and cardiac cells (Niebruegge *et al.*, 2009), chondrocytes (Koay and Athanasiou, 2008) and functional endothelium (Prado-Lopez *et al.*, 2010) from hESCs. Multiple studies have also been undertaken in self-renewing and differentiating hESCs/hiPSCs to identify differentially expressed proteins, which may then become targets for small molecule-mediated modulation (Chaerkady *et al.*, 2011; Gerwe *et al.*, 2011; Novak *et al.*, 2011; Kim *et al.*, 2011c).

Concluding remarks

The impact of small molecule compounds in hESC biology is hugely important, providing an effective and efficient means to maintain a pluripotent homogeneous starting cell population and promote specific differentiation. Further research promises to provide even more efficient and effective compounds and novel targets ultimately with the aim of providing useful therapeutic cells for cell replacement therapy.

Conflicts of interest

The authors declare no conflicts of interest.

References

Akopian V, Andrews PW, Beil S, Benvenisty N, Brehm J, Christie M *et al.* (2010). Comparison of defined culture systems for feeder cell free propagation of human embryonic stem cells. In Vitro Cell Dev Biol Anim 46: 247–258.

Alessi DR, James SR, Downes CP, Holmes AB, Gaffney PR, Reese CB *et al.* (1997). Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Balpha. Curr Biol 7: 261–269.

Andrews PD, Becroft M, Aspegren A, Gilmour J, James MJ, McRae S *et al.* (2010). High-content screening of feeder-free human embryonic stem cells to identify pro-survival small molecules. Biochem J 432: 21–33.

Anneren C, Cowan CA, Melton DA (2004). The Src family of tyrosine kinases is important for embryonic stem cell self-renewal. J Biol Chem 279: 31590–31598.

Armstrong L, Hughes O, Yung S, Hyslop L, Stewart R, Wappler I *et al.* (2006). The role of PI3K/AKT, MAPK/ERK and NFkappabeta signalling in the maintenance of human embryonic stem cell pluripotency and viability highlighted by transcriptional profiling and functional analysis. Hum Mol Genet 15: 1894–1913.

Bae D, Mondragon-Teran P, Hernandez D, Ruban L, Mason C, Bhattacharya SS *et al.* (2011). Hypoxia enhances the generation of retinal progenitor cells from human induced pluripotent and embryonic stem cells. Stem Cells Dev 21: 1344–1355.

Baharvand H, Salekdeh GH, Taei A, Mollamohammadi S (2010). An efficient and easy-to-use cryopreservation protocol for human ES and iPS cells. Nat Protoc 5: 588–594.

Barbaric I, Gokhale PJ, Andrews PW (2010a). High-content screening of small compounds on human embryonic stem cells. Biochem Soc Trans 38: 1046–1050.

Barbaric I, Gokhale PJ, Jones M, Glen A, Baker D, Andrews PW (2010b). Novel regulators of stem cell fates identified by a multivariate phenotype screen of small compounds on human embryonic stem cell colonies. Stem Cell Res 5: 104–119.

Barbaric I, Jones M, Buchner K, Baker D, Andrews PW, Moore HD (2011). Pinacidil enhances survival of cryopreserved human embryonic stem cells. Cryobiology 63: 298–305.

Beattie GM, Lopez AD, Bucay N, Hinton A, Firpo MT, King CC *et al*. (2005). Activin A maintains pluripotency of human embryonic stem cells in the absence of feeder layers. Stem Cells 23: 489–495.

Bendall SC, Stewart MH, Menendez P, George D, Vijayaragavan K, Werbowetski-Ogilvie T *et al*. (2007). IGF and FGF cooperatively establish the regulatory stem cell niche of pluripotent human cells in vitro. Nature 448: 1015–1021.

Bernardo AS, Faial T, Gardner L, Niakan KK, Ortmann D, Senner CE *et al*. (2011). BRACHYURY and CDX2 mediate BMP-induced differentiation of human and mouse pluripotent stem cells into embryonic and extraembryonic lineages. Cell Stem Cell 9: 144–155.

Bilousova G, Jun H, King KB, De Langhe S, Chick WS, Torchia EC *et al*. (2011). Osteoblasts derived from induced pluripotent stem cells form calcified structures in scaffolds both in vitro and in vivo. Stem Cells 29: 206–216.

Bissonnette CJ, Lyass L, Bhattacharyya BJ, Belmadani A, Miller RJ, Kessler JA (2011). The controlled generation of functional basal forebrain cholinergic neurons from human embryonic stem cells. Stem Cells 29: 802–811.

Bone HK, Damiano T, Bartlett S, Perry A, Letchford J, Ripoll YS *et al*. (2009). Involvement of GSK-3 in regulation of murine embryonic stem cell self-renewal revealed by a series of bisindolylmaleimides. Chem Biol 16: 15–27.

Bone HK, Nelson AS, Goldring CE, Tosh D, Welham MJ (2011). A novel chemically directed route for the generation of definitive endoderm from human embryonic stem cells based on inhibition of GSK-3. J Cell Sci 124 (Pt 12): 1992–2000.

Borowiak M, Maehr R, Chen S, Chen AE, Tang W, Fox JL *et al*. (2009). Small molecules efficiently direct endodermal differentiation of mouse and human embryonic stem cells. Cell Stem Cell 4: 348–358.

Bottcher RT, Niehrs C (2005). Fibroblast growth factor signaling during early vertebrate development. Endocr Rev 26: 63–77.

Braam SR, Nauw R, Ward-van Oostwaard D, Mummery C, Passier R (2010). Inhibition of ROCK improves survival of human embryonic stem cell-derived cardiomyocytes after dissociation. Ann N Y Acad Sci 1188: 52–57.

Brimble SN, Sherrer ES, Uhl EW, Wang E, Kelly S, Merrill AH Jr *et al*. (2007). The cell surface glycosphingolipids SSEA-3 and SSEA-4 are not essential for human ESC pluripotency. Stem Cells 25: 54–62.

Brons IG, Smithers LE, Trotter MW, Rugg-Gunn P, Sun B, Chuva de Sousa Lopes SM *et al*. (2007). Derivation of pluripotent epiblast stem cells from mammalian embryos. Nature 448: 191–195.

Brown S, Teo A, Pauklin S, Hannan N, Cho CH, Lim B *et al*. (2011). Activin/Nodal signaling controls divergent transcriptional networks in human embryonic stem cells and in endoderm progenitors. Stem Cells 29: 1176–1185.

Burdon T, Stracey C, Chambers I, Nichols J, Smith A (1999). Suppression of SHP-2 and ERK signalling promotes self-renewal of mouse embryonic stem cells. Dev Biol 210: 30–43.

Burka JM, Bower KS, Vanroekel RC, Stutzman RD, Kuzmowych CP, Howard RS (2005). The effect of fourth-generation fluoroquinolones gatifloxacin and moxifloxacin on epithelial healing following photorefractive keratectomy. Am J Ophthalmol 140: 83–87.

Burton P, Adams DR, Abraham A, Allcock RW, Jiang Z, McCahill A *et al*. (2010a). Identification and characterization of small-molecule ligands that maintain pluripotency of human embryonic stem cells. Biochem Soc Trans 38: 1058–1061.

Burton P, Adams DR, Abraham A, Allcock RW, Jiang Z, McCahill A *et al*. (2010b). Erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA) blocks differentiation and maintains the expression of pluripotency markers in human embryonic stem cells. Biochem J 432: 575–584.

Carson DA, Seegmiller JE (1976). Effect of adenosine deaminase inhibition upon human lymphocyte blastogenesis. J Clin Invest 57: 274–282.

Cezar GG, Quam JA, Smith AM, Rosa GJ, Piekarczyk MS, Brown JF *et al*. (2007). Identification of small molecules from human embryonic stem cells using metabolomics. Stem Cells Dev 16: 869–882.

Chaerkady R, Letzen B, Renuse S, Sahasrabuddhe NA, Kumar P, All AH *et al*. (2011). Quantitative temporal proteomic analysis of human embryonic stem cell differentiation into oligodendrocyte progenitor cells. Proteomics 11: 4007–4020.

Chambers SM, Fasano CA, Papapetrou EP, Tomishima M, Sadelain M, Studer L (2009). Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. Nat Biotechnol 27: 275–280.

Chang Y, Zhang X, Horton JR, Upadhyay AK, Spannhoff A, Liu J *et al*. (2009). Structural basis for G9a-like protein lysine methyltransferase inhibition by BIX-01294. Nat Struct Mol Biol 16: 312–317.

Chayosumrit M, Tuch B, Sidhu K (2010). Alginate microcapsule for propagation and directed differentiation of hESCs to definitive endoderm. Biomaterials 31: 505–514.

Chen B, Dodge ME, Tang W, Lu J, Ma Z, Fan CW *et al*. (2009a). Small molecule-mediated disruption of Wnt-dependent signaling in tissue regeneration and cancer. Nat Chem Biol 5: 100–107.

Chen G, Hou Z, Gulbranson DR, Thomson JA (2010a). Actin-myosin contractility is responsible for the reduced viability of dissociated human embryonic stem cells. Cell Stem Cell 7: 240–248.

Chen HF, Kuo HC, Lin SP, Chien CL, Chiang MS, Ho HN (2010b). Hypoxic culture maintains self-renewal and enhances embryoid body formation of human embryonic stem cells. Tissue Eng 16: 2901–2913.

Chen JK, Taipale J, Cooper MK, Beachy PA (2002). Inhibition of Hedgehog signaling by direct binding of cyclopamine to Smoothened. Genes Dev 16: 2743–2748.

Chen S, Borowiak M, Fox JL, Maehr R, Osafune K, Davidow L *et al*. (2009b). A small molecule that directs differentiation of human ESCs into the pancreatic lineage. Nat Chem Biol 5: 258–265.

Chng Z, Teo A, Pedersen RA, Vallier L (2011). SIP1 mediates cell-fate decisions between neuroectoderm and mesendoderm in human pluripotent stem cells. Cell Stem Cell 6: 59–70.

Christie VB, Barnard JH, Batsanov AS, Bridgens CE, Cartmell EB, Collings JC *et al*. (2008). Synthesis and evaluation of synthetic retinoid derivatives as inducers of stem cell differentiation. Org Biomol Chem 6: 3497–3507.

Chung S, Moon JI, Leung A, Aldrich D, Lukianov S, Kitayama Y *et al.* (2011). ES cell-derived renewable and functional midbrain dopaminergic progenitors. Proc Natl Acad Sci U S A 108: 9703–9708.

Conesa C, Doss MX, Antzelevitch C, Sachinidis A, Sancho J, Carrodeguas JA (2011). Identification of specific pluripotent stem cell death-inducing small molecules by chemical screening. Stem Cell Rev 8: 116–127.

Crabtree GR, Olson EN (2002). NFAT signaling: choreographing the social lives of cells. Cell 109 (Suppl.): S67–S79.

D'Amour KA, Agulnick AD, Eliazer S, Kelly OG, Kroon E, Baetge EE (2005). Efficient differentiation of human embryonic stem cells to definitive endoderm. Nat Biotechnol 23: 1534–1541.

D'Amour KA, Bang AG, Eliazer S, Kelly OG, Agulnick AD, Smart NG *et al.* (2006). Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. Nat Biotechnol 24: 1392–1401.

Darenfed H, Dayanandan B, Zhang T, Hsieh SH, Fournier AE, Mandato CA (2007). Molecular characterization of the effects of Y-27632. Cell Motil Cytoskeleton 64: 97–109.

Davie JR (2003). Inhibition of histone deacetylase activity by butyrate. J Nutr 133 (7 Suppl.): 2485S–2493S.

Desbordes SC, Placantonakis DG, Ciro A, Socci ND, Lee G, Djaballah H *et al.* (2008). High-throughput screening assay for the identification of compounds regulating self-renewal and differentiation in human embryonic stem cells. Cell Stem Cell 2: 602–612.

Dravid G, Ye Z, Hammond H, Chen G, Pyle A, Donovan P *et al.* (2005). Defining the role of Wnt/beta-catenin signaling in the survival, proliferation, and self-renewal of human embryonic stem cells. Stem Cells 23: 1489–1501.

Duester G (2008). Retinoic acid synthesis and signaling during early organogenesis. Cell 134: 921–931.

Emre N, Vidal JG, Elia J, O'Connor ED, Paramban RI, Hefferan MP *et al.* (2010). The ROCK inhibitor Y-27632 improves recovery of human embryonic stem cells after fluorescence-activated cell sorting with multiple cell surface markers. PLoS ONE 5: e12148.

Esteban MA, Wang T, Qin B, Yang J, Qin D, Cai J *et al.* (2010). Vitamin C enhances the generation of mouse and human induced pluripotent stem cells. Cell Stem Cell 6: 71–79.

Etienne-Manneville S, Hall A (2002). Rho GTPases in cell biology. Nature 420: 629–635.

Ezashi T, Das P, Roberts RM (2005). Low O2 tensions and the prevention of differentiation of hES cells. Proc Natl Acad Sci U S A 102: 4783–4788.

Forristal CE, Wright KL, Hanley NA, Oreffo RO, Houghton FD (2010). Hypoxia inducible factors regulate pluripotency and proliferation in human embryonic stem cells cultured at reduced oxygen tensions. Reproduction 139: 85–97.

Franke TF, Kaplan DR, Cantley LC, Toker A (1997). Direct regulation of the Akt proto-oncogene product by phosphatidylinositol-3,4-bisphosphate. Science 275: 665–668.

Fujiwara M, Yan P, Otsuji TG, Narazaki G, Uosaki H, Fukushima H *et al.* (2011). Induction and enhancement of cardiac cell differentiation from mouse and human induced pluripotent stem cells with cyclosporin-A. PLoS ONE 6: e16734.

Furue MK, Na J, Jackson JP, Okamoto T, Jones M, Baker D *et al.* (2008). Heparin promotes the growth of human embryonic stem cells in a defined serum-free medium. Proc Natl Acad Sci U S A 105: 13409–13414.

Garcia A, Cayla X, Guergnon J, Dessauge F, Hospital V, Rebollo MP *et al.* (2003). Serine/threonine protein phosphatases PP1 and PP2A are key players in apoptosis. Biochimie 85: 721–726.

Gaur M, Ritner C, Sievers R, Pedersen A, Prasad M, Bernstein HS *et al.* (2010). Timed inhibition of p38MAPK directs accelerated differentiation of human embryonic stem cells into cardiomyocytes. Cytotherapy 12: 807–817.

Gauthaman K, Richards M, Wong J, Bongso A (2007). Comparative evaluation of the effects of statins on human stem and cancer cells in vitro. Reprod Biomed Online 15: 566–581.

Gauthaman K, Manasi N, Bongso A (2009). Statins inhibit the growth of variant human embryonic stem cells and cancer cells in vitro but not normal human embryonic stem cells. Br J Pharmacol 157: 962–973.

Georgianna WE, Young DD (2011). Development and utilization of non-coding RNA-small molecule interactions. Org Biomol Chem 9: 7969–7978.

Gerwe BA, Angel PM, West FD, Hasneen K, Young A, Orlando R *et al.* (2011). Membrane proteomic signatures of karyotypically normal and abnormal human embryonic stem cell lines and derivatives. Proteomics 11: 2515–2527.

Ginis I, Luo Y, Miura T, Thies S, Brandenberger R, Gerecht-Nir S *et al.* (2004). Differences between human and mouse embryonic stem cells. Dev Biol 269: 360–380.

Gonzalez R, Lee JW, Snyder EY, Schultz PG (2011). Dorsomorphin promotes human embryonic stem cell self-renewal. Angew Chem Int Ed Engl 50: 3439–3441.

Gore A, Li Z, Fung HL, Young JE, Agarwal S, Antosiewicz-Bourget J *et al.* (2011). Somatic coding mutations in human induced pluripotent stem cells. Nature 471: 63–67.

Graichen R, Xu X, Braam SR, Balakrishnan T, Norfiza S, Sieh S *et al.* (2008). Enhanced cardiomyogenesis of human embryonic stem cells by a small molecular inhibitor of p38 MAPK. Differentiation 76: 357–370.

Green MD, Chen A, Nostro MC, d'Souza SL, Schaniel C, Lemischka IR *et al.* (2011). Generation of anterior foregut endoderm from human embryonic and induced pluripotent stem cells. Nat Biotechnol 29: 267–272.

Grover GJ (1997). Pharmacology of ATP-sensitive potassium channel (KATP) openers in models of myocardial ischemia and reperfusion. Can J Physiol Pharmacol 75: 309–315.

Guttman M, Donaghey J, Carey BW, Garber M, Grenier JK, Munson G *et al.* (2011). lincRNAs act in the circuitry controlling pluripotency and differentiation. Nature 477: 295–300.

Han Y, Miller A, Mangada J, Liu Y, Swistowski A, Zhan M *et al.* (2009). Identification by automated screening of a small molecule that selectively eliminates neural stem cells derived from hESCs but not dopamine neurons. PLoS ONE 4: e7155.

Hara A, Taguchi A, Aoki H, Hatano Y, Niwa M, Yamada Y *et al.* (2010). Folate antagonist, methotrexate induces neuronal differentiation of human embryonic stem cells transplanted into nude mouse retina. Neurosci Lett 477: 138–143.

Hay DC, Zhao D, Fletcher J, Hewitt ZA, McLean D, Urruticoechea-Uriguen A *et al.* (2008). Efficient differentiation of hepatocytes from human embryonic stem cells exhibiting markers recapitulating liver development in vivo. Stem Cells 26: 894–902.

Heo JS, Han HJ (2006). ATP stimulates mouse embryonic stem cell proliferation via protein kinase C, phosphatidylinositol 3-kinase/Akt, and mitogen-activated protein kinase signaling pathways. Stem Cells 24: 2637–2648.

Hirano K, Nagata S, Yamaguchi S, Nakagawa M, Okita K, Kotera H *et al*. (2011). Human and Mouse Induced Pluripotent Stem Cells Are Differentially Reprogrammed in response to Kinase Inhibitors. Stem Cells Dev 21: 1287–1298.

Hotta R, Pepdjonovic L, Anderson RB, Zhang D, Bergner AJ, Leung J *et al*. (2009). Small-molecule induction of neural crest-like cells derived from human neural progenitors. Stem Cells 27: 2896–2905.

Huangfu D, Osafune K, Maehr R, Guo W, Eijkelenboom A, Chen S *et al*. (2008). Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. Nat Biotechnol 26: 1269–1275.

Hudson JE, Titmarsh DM, Hidalgo A, Wolvetang EJ, Cooper-White JJ (2011). Primitive Cardiac Cells from Human Embryonic Stem Cells. Stem Cells Dev DOI: 10.1089/scd.2011.0254 [Epub ahead of print].

Humphrey RK, Beattie GM, Lopez AD, Bucay N, King CC, Firpo MT *et al*. (2004). Maintenance of pluripotency in human embryonic stem cells is STAT3 independent. Stem Cells 22: 522–530.

Hussein SM, Batada NN, Vuoristo S, Ching RW, Autio R, Narva E *et al*. (2011). Copy number variation and selection during reprogramming to pluripotency. Nature 471: 58–62.

Ilic D, Stephenson E, Wood V, Jacquet L, Stevenson D, Petrova A *et al*. (2011). Derivation and feeder-free propagation of human embryonic stem cells under xeno-free conditions. Cytotherapy 14: 122–128.

Ingham PW, McMahon AP (2001). Hedgehog signaling in animal development: paradigms and principles. Genes Dev 15: 3059–3087.

Inman GJ, Nicolas FJ, Callahan JF, Harling JD, Gaster LM, Reith AD *et al*. (2002). SB-431542 is a potent and specific inhibitor of transforming growth factor-beta superfamily type I activin receptor-like kinase (ALK) receptors ALK4, ALK5, and ALK7. Mol Pharmacol 62: 65–74.

Irie K, Nakahara A, Nakagawa Y, Ohigashi H, Shindo M, Fukuda H *et al*. (2002). Establishment of a binding assay for protein kinase C isozymes using synthetic C1 peptides and development of new medicinal leads with protein kinase C isozyme and C1 domain selectivity. Pharmacol Ther 93: 271–281.

Irwin EF, Gupta R, Dashti DC, Healy KE (2011). Engineered polymer-media interfaces for the long-term self-renewal of human embryonic stem cells. Biomaterials 32: 6912–6919.

James D, Levine AJ, Besser D, Hemmati-Brivanlou A (2005). TGFbeta/activin/nodal signaling is necessary for the maintenance of pluripotency in human embryonic stem cells. Development 132: 1273–1282.

James D, Nam HS, Seandel M, Nolan D, Janovitz T, Tomishima M *et al*. (2010). Expansion and maintenance of human embryonic stem cell-derived endothelial cells by TGFbeta inhibition is Id1 dependent. Nat Biotechnol 28: 161–166.

Ji AR, Ku SY, Cho MS, Kim YY, Kim YJ, Oh SK *et al*. (2010). Reactive oxygen species enhance differentiation of human embryonic stem cells into mesendodermal lineage. Exp Mol Med 42: 175–186.

Ji J, Ng SH, Sharma V, Neculai D, Hussein S, Sam M *et al*. (2012). Elevated coding mutation rate during the reprogramming of human somatic cells into induced pluripotent stem cells. Stem Cells 30: 435–440.

Jiang W, Shi Y, Zhao D, Chen S, Yong J, Zhang J *et al*. (2007). In vitro derivation of functional insulin-producing cells from human embryonic stem cells. Cell Res 17: 333–344.

Judson RL, Babiarz JE, Venere M, Blelloch R (2009). Embryonic stem cell-specific microRNAs promote induced pluripotency. Nat Biotechnol 27: 459–461.

Kamath AB, Wang L, Das H, Li L, Reinhold VN, Bukowski JF (2003). Antigens in tea-beverage prime human Vgamma 2Vdelta 2 T cells in vitro and in vivo for memory and nonmemory antibacterial cytokine responses. Proc Natl Acad Sci U S A 100: 6009–6014.

Kane NM, Meloni M, Spencer HL, Craig MA, Strehl R, Milligan G *et al*. (2010). Derivation of endothelial cells from human embryonic stem cells by directed differentiation: analysis of microRNA and angiogenesis in vitro and in vivo. Arterioscler Thromb Vasc Biol 30: 1389–1397.

Kane NM, Xiao Q, Baker AH, Luo Z, Xu Q, Emanueli C (2011). Pluripotent stem cell differentiation into vascular cells: a novel technology with promises for vascular re(generation). Pharmacol Ther 129: 29–49.

Kattman SJ, Witty AD, Gagliardi M, Dubois NC, Niapour M, Hotta A *et al*. (2011). Stage-specific optimization of activin/nodal and BMP signaling promotes cardiac differentiation of mouse and human pluripotent stem cell lines. Cell Stem Cell 8: 228–240.

Kelly TK, De Carvalho DD, Jones PA (2010). Epigenetic modifications as therapeutic targets. Nat Biotechnol 28: 1069–1078.

Kempf H, Lecina M, Ting S, Zweigerdt R, Oh S (2011). Distinct regulation of mitogen-activated protein kinase activities is coupled with enhanced cardiac differentiation of human embryonic stem cells. Stem Cell Res 7: 198–209.

Kim CG, Chung IY, Lim Y, Lee YH, Shin SY (2011a). A Tcf/Lef element within the enhancer region of the human NANOG gene plays a role in promoter activation. Biochem Biophys Res Commun 410: 637–642.

Kim DS, Lee JS, Leem JW, Huh YJ, Kim JY, Kim HS *et al*. (2010). Robust enhancement of neural differentiation from human ES and iPS cells regardless of their innate difference in differentiation propensity. Stem Cell Rev 6: 270–281.

Kim J, Su SC, Wang H, Cheng AW, Cassady JP, Lodato MA *et al*. (2011b). Functional integration of dopaminergic neurons directly converted from mouse fibroblasts. Cell Stem Cell 9: 413–419.

Kim SY, Kim MJ, Jung H, Kim WK, Kwon SO, Son MJ *et al*. (2011c). Comparative proteomic analysis of human somatic cells, induced pluripotent stem cells, and embryonic stem cells. Stem Cells Dev 21: 1272–1286.

Kim YY, Ku SY, Rosenwaks Z, Liu HC, Oh SK, Moon SY *et al*. (2011d). Red ginseng extract facilitates the early differentiation of human embryonic stem cells into mesendoderm lineage. Evid Based Complement Alternat Med 2011.

Klein PS, Melton DA (1996). A molecular mechanism for the effect of lithium on development. Proc Natl Acad Sci U S A 93: 8455–8459.

Klim JR, Li L, Wrighton PJ, Piekarczyk MS, Kiessling LL (2010). A defined glycosaminoglycan-binding substratum for human pluripotent stem cells. Nat Methods 7: 989–994.

Koay EJ, Athanasiou KA (2008). Hypoxic chondrogenic differentiation of human embryonic stem cells enhances cartilage protein synthesis and biomechanical functionality. Osteoarthritis Cartilage 16: 1450–1456.

Kobaek-Larsen M, Christensen LP, Vach W, Ritskes-Hoitinga J, Brandt K (2005). Inhibitory effects of feeding with carrots or (-)-falcarinol on development of azoxymethane-induced preneoplastic lesions in the rat colon. J Agric Food Chem 53: 1823–1827.

Kriks S, Shim JW, Piao J, Ganat YM, Wakeman DR, Xie Z *et al.* (2011). Dopamine neurons derived from human ES cells efficiently engraft in animal models of Parkinson's disease. Nature 480: 547–551.

Kroon E, Martinson LA, Kadoya K, Bang AG, Kelly OG, Eliazer S *et al.* (2008). Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. Nat Biotechnol 26: 443–452.

Kunath T, Saba-El-Leil MK, Almousailleakh M, Wray J, Meloche S, Smith A (2007). FGF stimulation of the Erk1/2 signalling cascade triggers transition of pluripotent embryonic stem cells from self-renewal to lineage commitment. Development 134: 2895–2902.

Kunisada Y, Tsubooka-Yamazoe N, Shoji M, Hosoya M (2011). Small molecules induce efficient differentiation into insulin-producing cells from human induced pluripotent stem cells. Stem Cell Res 8: 274–284.

Laping NJ, Grygielko E, Mathur A, Butter S, Bomberger J, Tweed C *et al.* (2002). Inhibition of transforming growth factor (TGF)-beta1-induced extracellular matrix with a novel inhibitor of the TGF-beta type I receptor kinase activity: SB-431542. Mol Pharmacol 62: 58–64.

Laurent LC, Ulitsky I, Slavin I, Tran H, Schork A, Morey R *et al.* (2011). Dynamic changes in the copy number of pluripotency and cell proliferation genes in human ESCs and iPSCs during reprogramming and time in culture. Cell Stem Cell 8: 106–118.

Lee DS, Yu K, Rho JY, Lee E, Han JS, Koo DB *et al.* (2006). Cyclopamine treatment of human embryonic stem cells followed by culture in human astrocyte medium promotes differentiation into nestin- and GFAP-expressing astrocytic lineage. Life Sci 80: 154–159.

Lee KW, Yook JY, Son MY, Kim MJ, Koo DB, Han YM *et al.* (2010). Rapamycin promotes the osteoblastic differentiation of human embryonic stem cells by blocking the mTOR pathway and stimulating the BMP/Smad pathway. Stem Cells Dev 19: 557–568.

Lee S, Kim J, Park TJ, Shin Y, Lee SY, Han YM *et al.* (2011). The effects of the physical properties of culture substrates on the growth and differentiation of human embryonic stem cells. Biomaterials 32: 8816–8829.

Li J, Wang G, Wang C, Zhao Y, Zhang H, Tan Z *et al.* (2007). MEK/ERK signaling contributes to the maintenance of human embryonic stem cell self-renewal. Differentiation 75: 299–307.

Li L, Wang BH, Wang S, Moalim-Nour L, Mohib K, Lohnes D *et al.* (2010). Individual cell movement, asymmetric colony expansion, rho-associated kinase, and E-cadherin impact the clonogenicity of human embryonic stem cells. Biophys J 98: 2442–2451.

Li W, Wei W, Zhu S, Zhu J, Shi Y, Lin T *et al.* (2009a). Generation of rat and human induced pluripotent stem cells by combining genetic reprogramming and chemical inhibitors. Cell Stem Cell 4: 16–19.

Li W, Zhou H, Abujarour R, Zhu S, Young Joo J, Lin T *et al.* (2009b). Generation of human-induced pluripotent stem cells in the absence of exogenous Sox2. Stem Cells 27: 2992–3000.

Li W, Sun W, Zhang Y, Wei W, Ambasudhan R, Xia P *et al.* (2011). Rapid induction and long-term self-renewal of primitive neural precursors from human embryonic stem cells by small molecule inhibitors. Proc Natl Acad Sci U S A 108: 8299–8304.

Li XJ, Hu BY, Jones SA, Zhang YS, Lavaute T, Du ZW *et al.* (2008). Directed differentiation of ventral spinal progenitors and motor neurons from human embryonic stem cells by small molecules. Stem Cells 26: 886–893.

Lim HJ, Han J, Woo DH, Kim SE, Kim SK, Kang HG *et al.* (2011). Biochemical and morphological effects of hypoxic environment on human embryonic stem cells in long-term culture and differentiating embryoid bodies. Mol Cells 31: 123–132.

Lim HW, De Windt LJ, Mante J, Kimball TR, Witt SA, Sussman MA *et al.* (2000). Reversal of cardiac hypertrophy in transgenic disease models by calcineurin inhibition. J Mol Cell Cardiol 32: 697–709.

Lin T, Ambasudhan R, Yuan X, Li W, Hilcove S, Abujarour R *et al.* (2009). A chemical platform for improved induction of human iPSCs. Nat Methods 6: 805–808.

Lister R, Pelizzola M, Kida YS, Hawkins RD, Nery JR, Hon G *et al.* (2011). Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. Nature 471: 68–73.

Liu L, Riese J, Resch K, Kaever V (1994). Impairment of macrophage eicosanoid and nitric oxide production by an alkaloid from Sinomenium acutum. Arzneimittelforschung 44: 1223–1226.

Lowenberg M, Stahn C, Hommes DW, Buttgereit F (2008). Novel insights into mechanisms of glucocorticoid action and the development of new glucocorticoid receptor ligands. Steroids 73: 1025–1029.

Ludwig TE, Levenstein ME, Jones JM, Berggren WT, Mitchen ER, Frane JL *et al.* (2006). Derivation of human embryonic stem cells in defined conditions. Nat Biotechnol 24: 185–187.

Mahmood A, Harkness L, Schroder HD, Abdallah BM, Kassem M (2010). Enhanced differentiation of human embryonic stem cells to mesenchymal progenitors by inhibition of TGF-beta/activin/nodal signaling using SB-431542. J Bone Miner Res 25: 1216–1233.

Maimets T, Neganova I, Armstrong L, Lako M (2008). Activation of p53 by nutlin leads to rapid differentiation of human embryonic stem cells. Oncogene 27: 5277–5287.

Maira SM, Stauffer F, Schnell C, Garcia-Echeverria C (2009). PI3K inhibitors for cancer treatment: where do we stand? Biochem Soc Trans 37 (Pt 1): 265–272.

Mali P, Chou BK, Yen J, Ye Z, Zou J, Dowey S *et al.* (2010). Butyrate greatly enhances derivation of human induced pluripotent stem cells by promoting epigenetic remodeling and the expression of pluripotency-associated genes. Stem Cells 28: 713–720.

Martin MJ, Muotri A, Gage F, Varki A (2005). Human embryonic stem cells express an immunogenic nonhuman sialic acid. Nat Med 11: 228–232.

Martin-Ibanez R, Unger C, Stromberg A, Baker D, Canals JM, Hovatta O (2008). Novel cryopreservation method for dissociated human embryonic stem cells in the presence of a ROCK inhibitor. Hum Reprod 23: 2744–2754.

Martins-Taylor K, Nisler BS, Taapken SM, Compton T, Crandall L, Montgomery KD *et al.* (2011). Recurrent copy number variations in human induced pluripotent stem cells. Nat Biotechnol 29: 488–491.

Matsubara K, Sanoh S, Ohta S, Kitamura S, Sugihara K, Fujimoto N (2011). An improved thyroid hormone reporter assay to determine the thyroid hormone-like activity of amiodarone, bithionol, closantel and rafoxanide. Toxicol Lett 208: 30–35.

Matsuda T, Nakamura T, Nakao K, Arai T, Katsuki M, Heike T *et al.* (1999). STAT3 activation is sufficient to maintain an undifferentiated state of mouse embryonic stem cells. EMBO J 18: 4261–4269.

Mazumdar J, O'Brien WT, Johnson RS, LaManna JC, Chavez JC, Klein PS *et al.* (2010). O2 regulates stem cells through Wnt/beta-catenin signalling. Nat Cell Biol 12: 1007–1013.

McLean AB, D'Amour KA, Jones KL, Krishnamoorthy M, Kulik MJ, Reynolds DM *et al*. (2007). Activin a efficiently specifies definitive endoderm from human embryonic stem cells only when phosphatidylinositol 3-kinase signaling is suppressed. Stem Cells 25: 29–38.

Mei Y, Saha K, Bogatyrev SR, Yang J, Hook AL, Kalcioglu ZI *et al*. (2010). Combinatorial development of biomaterials for clonal growth of human pluripotent stem cells. Nat Mater 9: 768–778.

Meijer L, Skaltsounis AL, Magiatis P, Polychronopoulos P, Knockaert M, Leost M *et al*. (2003). GSK-3-selective inhibitors derived from Tyrian purple indirubins. Chem Biol 10: 1255–1266.

Melkoumian Z, Weber JL, Weber DM, Fadeev AG, Zhou Y, Dolley-Sonneville P *et al*. (2010). Synthetic peptide-acrylate surfaces for long-term self-renewal and cardiomyocyte differentiation of human embryonic stem cells. Nat Biotechnol 28: 606–610.

Menendez L, Yatskievych TA, Antin PB, Dalton S (2011). Wnt signaling and a Smad pathway blockade direct the differentiation of human pluripotent stem cells to multipotent neural crest cells. Proc Natl Acad Sci U S A 108: 19240–19245.

Michie AM, Lobban M, Muller T, Harnett MM, Houslay MD (1996). Rapid regulation of PDE-2 and PDE-4 cyclic AMP phosphodiesterase activity following ligation of the T cell antigen receptor on thymocytes: analysis using the selective inhibitors erythro-9-(2-hydroxy-3-nonyl)-adenine (EHNA) and rolipram. Cell Signal 8: 97–110.

Montes R, Ligero G, Sanchez L, Catalina P, de la Cueva T, Nieto A *et al*. (2009). Feeder-free maintenance of hESCs in mesenchymal stem cell-conditioned media: distinct requirements for TGF-beta and IGF-II. Cell Res 19: 698–709.

Morizane A, Doi D, Kikuchi T, Nishimura K, Takahashi J (2011). Small-molecule inhibitors of bone morphogenic protein and activin/nodal signals promote highly efficient neural induction from human pluripotent stem cells. J Neurosci Res 89: 117–126.

Mullen AC, Orlando DA, Newman JJ, Lovén J, Kumar RM, Bilodeau S *et al*. (2011). Master Transcription Factors Determine Cell-Type-Specific Responses to TGF-^{2} Signaling. Cell 147: 565–576.

Müller M, Stockmann M, Malan D, Wolheim A, Tischendorf M, Linta L *et al*. (2011). Ca2+ Activated K Channels-New Tools to induce cardiac commitment from pluripotent stem cells in mice and men. Stem Cell Rev DOI: 10.1007/s12015-011-9324-9 [Epub ahead of print].

Mummery C, Ward-van Oostwaard D, Doevendans P, Spijker R, van den Brink S, Hassink R *et al*. (2003). Differentiation of human embryonic stem cells to cardiomyocytes: role of coculture with visceral endoderm-like cells. Circulation 107: 2733–2740.

Nandivada H, Villa-Diaz LG, O'Shea KS, Smith GD, Krebsbach PH, Lahann J (2011). Fabrication of synthetic polymer coatings and their use in feeder-free culture of human embryonic stem cells. Nat Protoc 6: 1037–1043.

Nathan PJ, Lu K, Gray M, Oliver C (2006). The neuropharmacology of L-theanine(N-ethyl-L-glutamine): a possible neuroprotective and cognitive enhancing agent. J Herb Pharmacother 6: 21–30.

Ng K-M, Chan Y-C, Lee Y-K, Lai W-H, Au K-W, Fung M-L *et al*. (2011). Cobalt chloride pretreatment promotes cardiac differentiation of human embryonic stem cells under atmospheric oxygen level. Cell Reprogram 13: 527–537.

Niebruegge S, Bauwens CL, Peerani R, Thavandiran N, Masse S, Sevaptisidis E *et al*. (2009). Generation of human embryonic stem cell-derived mesoderm and cardiac cells using size-specified aggregates in an oxygen-controlled bioreactor. Biotechnol Bioeng 102: 493–507.

Nissan X, Larribere L, Saidani M, Hurbain I, Delevoye C, Feteira J *et al*. (2011). Functional melanocytes derived from human pluripotent stem cells engraft into pluristratified epidermis. Proc Natl Acad Sci U S A 108: 14861–14866.

Niwa H, Burdon T, Chambers I, Smith A (1998). Self-renewal of pluripotent embryonic stem cells is mediated via activation of STAT3. Genes Dev 12: 2048–2060.

Nori S, Okada Y, Yasuda A, Tsuji O, Takahashi Y, Kobayashi Y *et al*. (2011). Grafted human-induced pluripotent stem-cell-derived neurospheres promote motor functional recovery after spinal cord injury in mice. Proc Natl Acad Sci U S A 108: 16825–16830.

Nostro MC, Sarangi F, Ogawa S, Holtzinger A, Corneo B, Li X *et al*. (2011). Stage-specific signaling through TGFbeta family members and WNT regulates patterning and pancreatic specification of human pluripotent stem cells. Development 138: 861–871.

Novak A, Amit M, Ziv T, Segev H, Fishman B, Admon A *et al*. (2011). Proteomics profiling of human embryonic stem cells in the early differentiation stage. Stem Cell Rev 8: 137–149.

Ohgushi M, Matsumura M, Eiraku M, Murakami K, Aramaki T, Nishiyama A *et al*. (2010). Molecular pathway and cell state responsible for dissociation-induced apoptosis in human pluripotent stem cells. Cell Stem Cell 7: 225–239.

Osakada F, Jin ZB, Hirami Y, Ikeda H, Danjyo T, Watanabe K *et al*. (2009). In vitro differentiation of retinal cells from human pluripotent stem cells by small-molecule induction. J Cell Sci 122 (Pt 17): 3169–3179.

Pakzad M, Totonchi M, Taei A, Seifinejad A, Hassani SN, Baharvand H (2010). Presence of a ROCK inhibitor in extracellular matrix supports more undifferentiated growth of feeder-free human embryonic and induced pluripotent stem cells upon passaging. Stem Cell Rev 6: 96–107.

Park SW, Jun Koh Y, Jeon J, Cho YH, Jang MJ, Kang Y *et al*. (2010). Efficient differentiation of human pluripotent stem cells into functional CD34+ progenitor cells by combined modulation of the MEK/ERK and BMP4 signaling pathways. Blood 116: 5762–5772.

Pasquale EB (2005). Eph receptor signalling casts a wide net on cell behaviour. Nat Rev Mol Cell Biol 6: 462–475.

Patani R, Compston A, Puddifoot CA, Wyllie DJ, Hardingham GE, Allen ND *et al*. (2009). Activin/Nodal inhibition alone accelerates highly efficient neural conversion from human embryonic stem cells and imposes a caudal positional identity. PLoS ONE 4: e7327.

Patani R, Hollins AJ, Wishart TM, Puddifoot CA, Alvarez S, de Lera AR *et al*. (2011). Retinoid-independent motor neurogenesis from human embryonic stem cells reveals a medial columnar ground state. Nat Commun 2: 214.

Pebay A, Wong RC, Pitson SM, Wolvetang EJ, Peh GS, Filipczyk A *et al*. (2005). Essential roles of sphingosine-1-phosphate and platelet-derived growth factor in the maintenance of human embryonic stem cells. Stem Cells 23: 1541–1548.

Prado-Lopez S, Conesa A, Arminan A, Martinez-Losa M, Escobedo-Lucea C, Gandia C *et al*. (2010). Hypoxia promotes efficient differentiation of human embryonic stem cells to functional endothelium. Stem Cells 28: 407–418.

Pyle AD, Lock LF, Donovan PJ (2006). Neurotrophins mediate human embryonic stem cell survival. Nat Biotechnol 24: 344–350.

Qi X, Li TG, Hao J, Hu J, Wang J, Simmons H *et al*. (2004). BMP4 supports self-renewal of embryonic stem cells by inhibiting mitogen-activated protein kinase pathways. Proc Natl Acad Sci U S A 101: 6027–6032.

Rai M, Walthall JM, Hu J, Hatzopoulos AK (2011). Continuous Antagonism by Dkk1 Counter Activates Canonical Wnt Signaling and Promotes Cardiomyocyte Differentiation of Embryonic Stem Cells. Stem Cells Dev 21: 54–66.

Ren Y, Lee MY, Schliffke S, Paavola J, Amos PJ, Ge X *et al*. (2011). Small molecule Wnt inhibitors enhance the efficiency of BMP-4-directed cardiac differentiation of human pluripotent stem cells. J Mol Cell Cardiol 51: 280–287.

Rezania A, Riedel MJ, Wideman RD, Karanu F, Ao Z, Warnock GL *et al*. (2011). Production of functional glucagon-secreting alpha-cells from human embryonic stem cells. Diabetes 60: 239–247.

Riento K, Ridley AJ (2003). Rocks: multifunctional kinases in cell behaviour. Nat Rev Mol Cell Biol 4: 446–456.

Rodin S, Domogatskaya A, Strom S, Hansson EM, Chien KR, Inzunza J *et al*. (2010). Long-term self-renewal of human pluripotent stem cells on human recombinant laminin-511. Nat Biotechnol 28: 611–615.

Rosen ED, Spiegelman BM (2000). Molecular regulation of adipogenesis. Annu Rev Cell Dev Biol 16: 145–171.

Ruegg UT, Burgess GM (1989). Staurosporine, K-252 and UCN-01: potent but nonspecific inhibitors of protein kinases. Trends Pharmacol Sci 10: 218–220.

Ruiz i Altaba A (1999). Gli proteins encode context-dependent positive and negative functions: implications for development and disease. Development 126: 3205–3216.

Saha K, Mei Y, Reisterer CM, Pyzocha NK, Yang J, Muffat J *et al*. (2011). Surface-engineered substrates for improved human pluripotent stem cell culture under fully defined conditions. Proc Natl Acad Sci U S A 108: 18714–18719.

Salli U, Fox TE, Carkaci-Salli N, Sharma A, Robertson GP, Kester M *et al*. (2009). Propagation of undifferentiated human embryonic stem cells with nano-liposomal ceramide. Stem Cells Dev 18: 55–65.

Sasaki N, Hirano T, Ichimiya T, Wakao M, Hirano K, Kinoshita-Toyoda A *et al*. (2009). The 3′-phosphoadenosine 5′-phosphosulfate transporters, PAPST1 and 2, contribute to the maintenance and differentiation of mouse embryonic stem cells. PLoS ONE 4: e8262.

Sasaki N, Hirano T, Kobayashi K, Toyoda M, Miyakawa Y, Okita H *et al*. (2010). Chemical inhibition of sulfation accelerates neural differentiation of mouse embryonic stem cells and human induced pluripotent stem cells. Biochem Biophys Res Commun 401: 480–486.

Sathananthan H, Pera M, Trounson A (2002). The fine structure of human embryonic stem cells. Reprod Biomed Online 4: 56–61.

Sato N, Meijer L, Skaltsounis L, Greengard P, Brivanlou AH (2004). Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. Nat Med 10: 55–63.

Seiffert D, Bradley JD, Rominger CM, Rominger DH, Yang F, Meredith JE Jr *et al*. (2000). Presenilin-1 and -2 are molecular targets for gamma-secretase inhibitors. J Biol Chem 275: 34086–34091.

Serena E, Figallo E, Tandon N, Cannizzaro C, Gerecht S, Elvassore N *et al*. (2009). Electrical stimulation of human embryonic stem cells: cardiac differentiation and the generation of reactive oxygen species. Exp Cell Res 315: 3611–3619.

Sinha S, Chen JK (2006). Purmorphamine activates the Hedgehog pathway by targeting Smoothened. Nat Chem Biol 2: 29–30.

Smith JR, Vallier L, Lupo G, Alexander M, Harris WA, Pedersen RA (2008). Inhibition of Activin/Nodal signaling promotes specification of human embryonic stem cells into neuroectoderm. Dev Biol 313: 107–117.

Smukler SR, Runciman SB, Xu S, van der Kooy D (2006). Embryonic stem cells assume a primitive neural stem cell fate in the absence of extrinsic influences. J Cell Biol 172: 79–90.

Taapken SM, Nisler BS, Newton MA, Sampsell-Barron TL, Leonhard KA, McIntire EM *et al*. (2011). Karotypic abnormalities in human induced pluripotent stem cells and embryonic stem cells. Nat Biotechnol 29: 313–314.

Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K *et al*. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131: 861–872.

Tejedo JR, Tapia-Limonchi R, Mora-Castilla S, Cahuana GM, Hmadcha A, Martin F *et al*. (2010). Low concentrations of nitric oxide delay the differentiation of embryonic stem cells and promote their survival. Cell Death Dis 1: e80.

Teo AK, Arnold SJ, Trotter MW, Brown S, Ang LT, Chng Z *et al*. (2011). Pluripotency factors regulate definitive endoderm specification through eomesodermin. Genes Dev 25: 238–250.

Tesar PJ, Chenoweth JG, Brook FA, Davies TJ, Evans EP, Mack DL *et al*. (2007). New cell lines from mouse epiblast share defining features with human embryonic stem cells. Nature 448: 196–199.

Thakur RK, Kumar P, Halder K, Verma A, Kar A, Parent JL *et al*. (2009). Metastases suppressor NM23-H2 interaction with G-quadruplex DNA within c-MYC promoter nuclease hypersensitive element induces c-MYC expression. Nucleic Acids Res 37: 172–183.

Thatava T, Nelson TJ, Edukulla R, Sakuma T, Ohmine S, Tonne JM *et al*. (2011). Indolactam V/GLP-1-mediated differentiation of human iPS cells into glucose-responsive insulin-secreting progeny. Gene Ther 18: 283–293.

Thomas JR, Hergenrother PJ (2008). Targeting RNA with small molecules. Chem Rev 108: 1171–1224.

Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS *et al*. (1998). Embryonic stem cell lines derived from human blastocysts. Science 282: 1145–1147.

Tiscornia G, Izpisua Belmonte JC (2010). MicroRNAs in embryonic stem cell function and fate. Genes Dev 24: 2732–2741.

Touboul T, Hannan NR, Corbineau S, Martinez A, Martinet C, Branchereau S *et al*. (2010). Generation of functional hepatocytes from human embryonic stem cells under chemically defined conditions that recapitulate liver development. Hepatology 51: 1754–1765.

Tsutsui H, Valamehr B, Hindoyan A, Qiao R, Ding X, Guo S *et al*. (2011). An optimized small molecule inhibitor cocktail supports long-term maintenance of human embryonic stem cells. Nat Commun 2: 167.

Tucker BA, Park IH, Qi SD, Klassen HJ, Jiang C, Yao J *et al*. (2011). Transplantation of adult mouse iPS cell-derived photoreceptor precursors restores retinal structure and function in degenerative mice. PLoS ONE 6: e18992.

Vallier L, Alexander M, Pedersen RA (2005). Activin/Nodal and FGF pathways cooperate to maintain pluripotency of human embryonic stem cells. J Cell Sci 118 (Pt 19): 4495–4509.

Vallier L, Mendjan S, Brown S, Chng Z, Teo A, Smithers LE *et al*. (2009a). Activin/Nodal signalling maintains pluripotency by controlling Nanog expression. Development 136: 1339–1349.

Vallier L, Touboul T, Brown S, Cho C, Bilican B, Alexander M *et al*. (2009b). Signaling pathways controlling pluripotency and early cell fate decisions of human induced pluripotent stem cells. Stem Cells 27: 2655–2666.

Vallier L, Touboul T, Chng Z, Brimpari M, Hannan N, Millan E *et al*. (2009c). Early cell fate decisions of human embryonic stem cells and mouse epiblast stem cells are controlled by the same signalling pathways. PLoS ONE 4: e6082.

Vanhaecke T, Papeleu P, Elaut G, Rogiers V (2004). Trichostatin A-like hydroxamate histone deacetylase inhibitors as therapeutic agents: toxicological point of view. Curr Med Chem 11: 1629–1643.

Varum S, Momcilovic O, Castro C, Ben-Yehudah A, Ramalho-Santos J, Navara CS (2009). Enhancement of human embryonic stem cell pluripotency through inhibition of the mitochondrial respiratory chain. Stem Cell Res 3: 142–156.

Villa-Diaz LG, Nandivada H, Ding J, Nogueira-de-Souza NC, Krebsbach PH, O'Shea KS *et al*. (2010). Synthetic polymer coatings for long-term growth of human embryonic stem cells. Nat Biotechnol 28: 581–583.

Vlahos CJ, Matter WF, Hui KY, Brown RF (1994). A specific inhibitor of phosphatidylinositol 3-kinase, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002). J Biol Chem 269: 5241–5248.

Wada T, Honda M, Minami I, Tooi N, Amagai Y, Nakatsuji N *et al*. (2009). Highly efficient differentiation and enrichment of spinal motor neurons derived from human and monkey embryonic stem cells. PLoS ONE 4: e6722.

Wang C, Tang X, Sun X, Miao Z, Lv Y, Yang Y *et al*. (2011a). TGFbeta inhibition enhances the generation of hematopoietic progenitors from human ES cell-derived hemogenic endothelial cells using a stepwise strategy. Cell Res 22: 194–207.

Wang Q, Xu X, Li J, Liu J, Gu H, Zhang R *et al*. (2011b). Lithium, an anti-psychotic drug, greatly enhances the generation of induced pluripotent stem cells. Cell Res 21: 1424–1435.

Wang Y, Baskerville S, Shenoy A, Babiarz JE, Baehner L, Blelloch R (2008). Embryonic stem cell-specific microRNAs regulate the G1-S transition and promote rapid proliferation. Nat Genet 40: 1478–1483.

Wang Y, Chen G, Song T, Mao G, Bai H (2010). Enhancement of cardiomyocyte differentiation from human embryonic stem cells. Sci China Life Sci 53: 581–589.

Ward CM, Eastham AM, Stern PL (2006). Cell surface 5T4 antigen is transiently upregulated during early human embryonic stem cell differentiation: effect of 5T4 phenotype on neural lineage formation. Exp Cell Res 312: 1713–1726.

Ware CB, Wang L, Mecham BH, Shen L, Nelson AM, Bar M *et al*. (2009). Histone deacetylase inhibition elicits an evolutionarily conserved self-renewal program in embryonic stem cells. Cell Stem Cell 4: 359–369.

Watanabe K, Ueno M, Kamiya D, Nishiyama A, Matsumura M, Wataya T *et al*. (2007). A ROCK inhibitor permits survival of dissociated human embryonic stem cells. Nat Biotechnol 25: 681–686.

Watashi K, Yeung ML, Starost MF, Hosmane RS, Jeang KT (2010). Identification of small molecules that suppress microRNA function and reverse tumorigenesis. J Biol Chem 285: 24707–24716.

Westfall SD, Sachdev S, Das P, Hearne LB, Hannink M, Roberts RM *et al*. (2008). Identification of oxygen-sensitive transcriptional programs in human embryonic stem cells. Stem Cells Dev 17: 869–881.

Willems E, Spiering S, Davidovics H, Lanier M, Xia Z, Dawson M *et al*. (2011). Small-molecule inhibitors of the wnt pathway potently promote cardiomyocytes from human embryonic stem cell-derived mesoderm. Circ Res 109: 360–364.

Wong RC, Pebay A, Nguyen LT, Koh KL, Pera MF (2004). Presence of functional gap junctions in human embryonic stem cells. Stem Cells 22: 883–889.

Xiao L, Yuan X, Sharkis SJ (2006). Activin A maintains self-renewal and regulates fibroblast growth factor, Wnt, and bone morphogenic protein pathways in human embryonic stem cells. Stem Cells 24: 1476–1486.

Xiong C, Xie CQ, Zhang L, Zhang J, Xu K, Fu M *et al*. (2005). Derivation of adipocytes from human embryonic stem cells. Stem Cells Dev 14: 671–675.

Xu C, Police S, Rao N, Carpenter MK (2002). Characterization and enrichment of cardiomyocytes derived from human embryonic stem cells. Circ Res 91: 501–508.

Xu RH, Peck RM, Li DS, Feng X, Ludwig T, Thomson JA (2005). Basic FGF and suppression of BMP signaling sustain undifferentiated proliferation of human ES cells. Nat Methods 2: 185–190.

Xu RH, Sampsell-Barron TL, Gu F, Root S, Peck RM, Pan G *et al*. (2008a). NANOG is a direct target of TGFbeta/activin-mediated SMAD signaling in human ESCs. Cell Stem Cell 3: 196–206.

Xu XQ, Graichen R, Soo SY, Balakrishnan T, Rahmat SN, Sieh S *et al*. (2008b). Chemically defined medium supporting cardiomyocyte differentiation of human embryonic stem cells. Differentiation 76: 958–970.

Xu Y, Zhu X, Hahm HS, Wei W, Hao E, Hayek A *et al*. (2010). Revealing a core signaling regulatory mechanism for pluripotent stem cell survival and self-renewal by small molecules. Proc Natl Acad Sci U S A 107: 8129–8134.

Yamasaki H (1976). Pharmacology of sinomenine, an anti-rheumatic alkaloid from Sinomenium acutum. Acta Med Okayama 30: 1–20.

Yanes O, Clark J, Wong DM, Patti GJ, Sanchez-Ruiz A, Benton HP *et al*. (2010). Metabolic oxidation regulates embryonic stem cell differentiation. Nat Chem Biol 6: 411–417.

Yang CS, Lopez CG, Rana TM (2011). Discovery of nonsteroidal anti-inflammatory drug and anticancer drug enhancing reprogramming and induced pluripotent stem cell generation. Stem Cells 29: 1528–1536.

Yi R, Fuchs E (2011). MicroRNAs and their roles in mammalian stem cells. J Cell Sci 124: 1775–1783.

Ying QL, 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 115: 281–292.

Ying QL, Wray J, Nichols J, Batlle-Morera L, Doble B, Woodgett J *et al*. (2008). The ground state of embryonic stem cell self-renewal. Nature 453: 519–523.

Yoon BS, Yoo SJ, Lee JE, You S, Lee HT, Yoon HS (2006). Enhanced differentiation of human embryonic stem cells into cardiomyocytes by combining hanging drop culture and 5-azacytidine treatment. Differentiation 74: 149–159.

Yoon BS, Jun EK, Park G, Jun Yoo S, Moon JH, Soon Baik C *et al*. (2010). Optimal suppression of protein phosphatase 2A activity is critical for maintenance of human embryonic stem cell self-renewal. Stem Cells 28: 874–884.

Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S *et al*. (2007). Induced pluripotent stem cell lines derived from human somatic cells. Science 318: 1917–1920.

Yu J, Huang NF, Wilson KD, Velotta JB, Huang M, Li Z *et al*. (2009). nAChRs mediate human embryonic stem cell-derived endothelial cells: proliferation, apoptosis, and angiogenesis. PLoS ONE 4: e7040.

Yu J, Chau KF, Vodyanik MA, Jiang J, Jiang Y (2011). Efficient feeder-free episomal reprogramming with small molecules. PLoS ONE 6: e17557.

Yu PB, Hong CC, Sachidanandan C, Babitt JL, Deng DY, Hoyng SA *et al*. (2008). Dorsomorphin inhibits BMP signals required for embryogenesis and iron metabolism. Nat Chem Biol 4: 33–41.

Yung S, Ledran M, Moreno-Gimeno I, Conesa A, Montaner D, Dopazo J *et al*. (2011). Large-scale transcriptional profiling and functional assays reveal important roles for Rho-GTPase signalling and SCL during haematopoietic differentiation of human embryonic stem cells. Hum Mol Genet 20: 4932–4934.

Zhang Q, Jiang J, Han P, Yuan Q, Zhang J, Zhang X *et al*. (2011). Direct differentiation of atrial and ventricular myocytes from human embryonic stem cells by alternating retinoid signals. Cell Res 21: 579–587.

Zhou AD, Diao LT, Xu H, Xiao ZD, Li JH, Zhou H *et al*. (2011). beta-Catenin/LEF1 transactivates the microRNA-371-373 cluster that modulates the Wnt/beta-catenin-signaling pathway. Oncogene DOI: 10.1038/onc.2011.461 [Epub ahead of print].

Zhou J, Su P, Wang L, Chen J, Zimmermann M, Genbacev O *et al*. (2009). mTOR supports long-term self-renewal and suppresses mesoderm and endoderm activities of human embryonic stem cells. Proc Natl Acad Sci U S A 106: 7840–7845.

Zhou J, Su P, Li D, Tsang S, Duan E, Wang F (2010). High-efficiency induction of neural conversion in human ESCs and human induced pluripotent stem cells with a single chemical inhibitor of transforming growth factor beta superfamily receptors. Stem Cells 28: 1741–1750.

Zhu MX, Zhao JY, Chen GA, Guan L (2011). Early embryonic sensitivity to cyclophosphamide in cardiac differentiation from human embryonic stem cells. Cell Biol Int 35: 927–938.

Zhu S, Wurdak H, Wang J, Lyssiotis CA, Peters EC, Cho CY *et al*. (2009). A small molecule primes embryonic stem cells for differentiation. Cell Stem Cell 4: 416–426.

Zhu S, Li W, Zhou H, Wei W, Ambasudhan R, Lin T *et al*. (2010). Reprogramming of human primary somatic cells by OCT4 and chemical compounds. Cell Stem Cell 7: 651–655.