The Effects of Genetic and Epigenetic Variation on Human Pluripotent Stem Cell Differentiation

Moraghebi, Roksana

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The Effects of Genetic and Epigenetic Variation on Human Pluripotent Stem Cell Differentiation
The Effects of Genetic and Epigenetic Variation on Human Pluripotent Stem Cell Differentiation

Roksana Moraghebi

DOCTORAL DISSERTATION
With the approval of the Faculty of Medicine, Lund University, this thesis will be defended on September 14th 2017 at 9:00 in Belfrage lecture hall, BMC D15, Klinikgatan 32, Lund, Sweden.

supervisor: Niels-Bjarne Woods

Faculty opponent
Professor Hanna Mikkola, M.D., Ph.D.
Abstract

Human pluripotent stem cells (PSCs) are widely used for studying embryonic development, disease modelling, drug discovery and cell therapy development. Using human PSCs as a model has significantly contributed to our understanding of embryonic development and elucidating novel pathological mechanisms as well as developing new drugs. However, there are significant genetic and epigenetic variations among hPSCs which can potentially affect their utility and differentiation potential, and thus undermine their applicability for downstream applications. In addition, female hPSCs under conventional culture conditions are epigenetically unstable, which is a major concern for disease modeling. The heterogeneity among hPSCs may be inherited from source cell populations, introduced during reprogramming or accumulated during culture. Regardless of the source of this heterogeneity and instability, if human PSCs are to be used in embryonic development and disease modeling studies or regenerative medicine, these variations need to be understood and perhaps adjusted for. Human PSCs can potentially differentiate into all cells of the body including hematopoietic cells, and thus hold great promise for hematopoietic stem cell transplantation or transfusion therapies. However, the heterogeneity among hPSCs can affect their hematopoietic differentiation potential. For example, the level of IGF2 expression in hiPSCs is correlated with their hematopoietic commitment capacity, and diversity in DNA methylation patterns of hiPSCs is associated with their hematopoietic maturation capacity.

It has been reported that differentiation potential of iPSC lines is skewed in favor of their source cell lineage. For example, iPSC lines derived from blood cells have a greater ability to differentiate towards blood cell lineages compared to the lines that are derived from skin cells. In paper I, we evaluate the influence of donor and cell type on the epigenome and hematopoietic differentiation potential of iPSC lines derived from blood and fibroblast cells of multiple donors. We demonstrate that donor genetic background has a much greater influence on the epigenome and hematopoietic differentiation potential of iPSC lines. Due to the large impact of donor on differentiation potential, in paper II we propose the establishment of a broad spectrum HLA type-matched iPSC bank derived from a broad donor base of term amniotic fluid derived cells in connection to planned caesarean section deliveries. Term amniotic fluid cells, being from a neonatal source have likely accumulated fewer genetic mutations compared to adult sources and thus are a better cell source for iPSC generation and will reduce variations among hiPSC lines. It has been also suggested that erosion of the transcriptionally inactive X chromosome in female PSCs under conventional culture condition is correlated with poor differentiation propensity, including hematopoietic differentiation potential. In paper III, we evaluate the effects of epigenetic instability of female hiPSC on their hematopoietic differentiation potential. We demonstrate that extensive erosion of the inactive X chromosome has a negative impact on hematopoiesis, and suggest a role for the X-linked gene ZIC3 in mediating the hematopoietic differentiation defect via Wnt signaling.

Key words: Pluripotent stem cells, genetic and epigenetic variation, developmental hematopoiesis, in vitro differentiation

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The Effects of Genetic and Epigenetic Variation on Human Pluripotent Stem Cell Differentiation

Roksana Moraghebi
To my family

تقديم به خانواده ی عزیزم
“Logic will get you from A to B. Imagination will take you everywhere”

-Albert Einstein
Content

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Original papers included in this thesis

**Paper I.** *Genetic Variability Overrides the Impact of Parental Cell Type and Determines iPSC Differentiation Potential.*

Aija Kyttälä, **Roksana Moraghebi**, Cristina Valensisi, Johannes Kettunen, Colin Andrus, Kalyan Kumar Pasumarthy, Mahito Nakanishi, Ken Nishimura, Manami Ohtaka, Jere Weltner, Ben Van Handel, Olavi Parkkonen, Juha Sinisalo, Anu Jalanko, R. David Hawkins, Niels-Bjarne Woods, Timo Otonkoski*, and Ras Trokovic*

Stem Cell Reports 2016 Feb 9;6(2):200-12.

**Paper II.** *Term amniotic fluid: an unexploited reserve of mesenchymal stromal cells for reprogramming and potential cell therapy applications.*

**Roksana Moraghebi**, Agnete Kirkeby, Patricia Chaves, Roger E. Rönn, Ewa Sitnicka, Malin Parmar, Marcus Larsson*, Andreas Herbst*, and Niels-Bjarne Woods*

Stem Cell Research and Therapy

**Paper III.** *Epigenetic variation of X chromosome inactivation in female human pluripotent stem cells is correlated with differentiation propensity.*

**Roksana Moraghebi**, Ben Van Handel, Sanjeet Patel, Xiaojie Xian, Agnete Kirkeby, Malin Parmar, Jonas Larsson, Kathrin Plath, and Niels-Bjarne Woods*

Manuscript

* Corresponding author
Papers not included in this thesis

*Corresponding author
Abbreviations

AGM  Aorta-gonad-mesonephros
BL-CFCs  Blast-colony forming cells
BM  Bone marrow
BMP4  Bone Morphogenetic Protein 4
CFU-C  Colony forming unit-cell
CNV  Copy number variation
CS  Carnegie stage
DNMT3B  DNA Methyltransferase 3 Beta
E  Embryonic day
EHT  Endothelial to hematopoietic transition
EMP  Erythroid-myeloid progenitors
EPI  Epiblast
EpiSC  Epiblast-derived stem cell
ERG  ETS-related gene
ESC  Embryonic stem cell
FGF  Fibroblast growth factor
Flk-1  Fetal liver kinase-1
FOXF1  Forkhead box o 1
GATA1  Globin transcription factor-1
GDF3  Growth differentiation factor 3
HE  Hemogenic endothelium
HOXA  Homeobox A cluster
hPSC  Human pluripotent stem cells
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Name</th>
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<tr>
<td>HSC</td>
<td>Hematopoietic stem cell</td>
</tr>
<tr>
<td>HUWE1</td>
<td>HECT, UBA And WWE Domain Containing 1, E3 Ubiquitin Protein Ligase</td>
</tr>
<tr>
<td>IAHC</td>
<td>Intra-aortic hematopoietic cluster</td>
</tr>
<tr>
<td>ICM</td>
<td>Inner cell mass</td>
</tr>
<tr>
<td>iPSC</td>
<td>Induced pluripotent stem cell</td>
</tr>
<tr>
<td>KDR</td>
<td>Kinase Insert Domain Receptor</td>
</tr>
<tr>
<td>KLF4</td>
<td>Kruppel Like Factor 4</td>
</tr>
<tr>
<td>LCOR</td>
<td>Ligand dependent nuclear receptor corepressor</td>
</tr>
<tr>
<td>LIN28</td>
<td>Lin-28 homolog A</td>
</tr>
<tr>
<td>IncRNA</td>
<td>Long non-coding RNA</td>
</tr>
<tr>
<td>MEF</td>
<td>Mouse embryonic fibroblast</td>
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<tr>
<td>mESC</td>
<td>Mouse embryonic stem cell</td>
</tr>
<tr>
<td>MIXL1</td>
<td>Mix Paired-Like Homeobox</td>
</tr>
<tr>
<td>NK</td>
<td>Natural killer</td>
</tr>
<tr>
<td>NOD-SCID</td>
<td>None-obese diabetic-sever combined immune deficiency</td>
</tr>
<tr>
<td>Notch</td>
<td>Neurogenic Locus Notch Homolog Protein</td>
</tr>
<tr>
<td>OCT4</td>
<td>Octamer-Binding Transcription Factor 4</td>
</tr>
<tr>
<td>PE</td>
<td>Primitive endoderm</td>
</tr>
<tr>
<td>PSC</td>
<td>Pluripotent stem cell</td>
</tr>
<tr>
<td>RA</td>
<td>Retinoic acid</td>
</tr>
<tr>
<td>Runx1</td>
<td>Runt-related transcription factor 1</td>
</tr>
<tr>
<td>SNV</td>
<td>Single nucleotide variation</td>
</tr>
<tr>
<td>SOX2</td>
<td>SRY-Box 2</td>
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<td>SPI1</td>
<td>Spi-1 Proto-Oncogene</td>
</tr>
<tr>
<td>TCF</td>
<td>T cell factor</td>
</tr>
<tr>
<td>TE</td>
<td>Trophoblast</td>
</tr>
<tr>
<td>TGFß</td>
<td>Transforming growth factor β</td>
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<tr>
<td>Term</td>
<td>Definition</td>
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<tr>
<td>-------</td>
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</tr>
<tr>
<td>UCB</td>
<td>Umbilical cord blood</td>
</tr>
<tr>
<td>Xa</td>
<td>Active X chromosome</td>
</tr>
<tr>
<td>XCD</td>
<td>X chromosome dampening</td>
</tr>
<tr>
<td>XCI</td>
<td>X chromosome inactivation</td>
</tr>
<tr>
<td>Xi</td>
<td>Inactive X chromosome</td>
</tr>
<tr>
<td>XIST</td>
<td>X Inactive Specific Transcript</td>
</tr>
<tr>
<td>Xme</td>
<td>Moderately eroded X chromosome</td>
</tr>
<tr>
<td>Xve</td>
<td>Vastly eroded X chromosome</td>
</tr>
<tr>
<td>ZIC3</td>
<td>Zic Family Member 3</td>
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Abstract

The discovery of induced pluripotent stem cells (iPSCs) has revolutionized the field of stem cell biology and enabled researchers to generate unlimited supplies of patient-specific cells with pluripotent potential similar to that of embryonic stem cells. Human pluripotent stem cells (PSCs) are widely used for studying embryonic development, disease modelling, drug discovery and cell therapy development. Using human PSCs as a model has significantly contributed to our understanding of embryonic development and elucidating novel pathological mechanisms as well as developing new drugs. However, there are significant genetic and epigenetic variations among hPSCs which can potentially affect their utility and differentiation potential, and thus undermine their applicability for downstream applications. In addition, female hPSCs under conventional culture conditions are epigenetically unstable, which is a major concern for disease modeling. The heterogeneity among hPSCs may be inherited from source cell populations, introduced during reprogramming or accumulated during culture. Regardless of the source of this heterogeneity and instability, if human PSCs are to be used in embryonic development and disease modeling studies or regenerative medicine, these variations need to be understood and perhaps adjusted for.

Human PSCs can potentially differentiate into all cells of the body including hematopoietic cells, and thus hold great promise for hematopoietic stem cell (Ross et al.) transplantation or transfusion therapies. However, the heterogeneity among hPSCs can affect their hematopoietic differentiation potential. For example, the level of IGF2 expression in hiPSCs is correlated with their hematopoietic commitment capacity, and diversity in DNA methylation patterns of hiPSCs is associated with their hematopoietic maturation capacity.

It has been reported that differentiation potential of iPSC lines is skewed in favor of their source cell lineage (Kim et al., 2010, Polo et al., 2010, Bar-Nur et al., 2011, Kim et al., 2011, Lister et al., 2011, Ruiz et al., 2012). For example, iPSC lines derived from blood cells have a greater ability to differentiate towards blood cell lineages compared to the lines that are derived from skin cells (Kim et al., 2011). In paper I, we evaluate the influence of donor and cell type on the epigenome and
hematopoietic differentiation potential of iPSC lines derived from blood and fibroblast cells of multiple donors. We demonstrate that donor genetic background has a much greater influence on the epigenome and hematopoietic differentiation potential of iPSC lines. Due to the large impact of donor on differentiation potential, in paper II we propose the establishment of a broad spectrum HLA type-matched iPSC bank derived from a broad donor base of term amniotic fluid derived cells in connection to planned caesarean section deliveries. Term amniotic fluid cells, being from a neonatal source have likely accumulated fewer genetic mutations compared to adult sources and thus are a better cell source for iPSC generation and will reduce variations among hiPSC lines. It has been also suggested that erosion of the transcriptionally inactive X chromosome in female PSCs under conventional culture condition is correlated with poor differentiation propensity, including hematopoietic differentiation potential (Patel et al., 2017). In paper III, we evaluate the effects of epigenetic instability of female hiPSC on their hematopoietic differentiation potential. We demonstrate that extensive erosion of the inactive X chromosome has a negative impact on hematopoiesis, and suggest a role for the X-linked gene ZIC3 in mediating the hematopoietic differentiation defect via Wnt signaling.
1. Stem cells

Stem cells are highly plastic cells that are capable of both self-renewal and differentiating into specialized cell types. There are two broad types of stem cells: embryonic stem cells which are derived from inner cell mass (ICM) of embryos, and adult stem cells which can be isolated from various adult tissues. Stem cells are classified into five different categories based on their differentiation potential: totipotent, pluripotent, multipotent, oligopotent and unipotent stem cells. Totipotent stem cells reside at the top of hierarchy of cell potency with the most differentiation potential, and unipotent stem cells are at the bottom of hierarchy and can produce only one cell type. Totipotent cells have the potential to give rise to both embryonic and extra-embryonic tissues. Pluripotency stem cells are descendants of totipotent stem cells and are capable of developing into any of the three germ cell layers (endoderm, mesoderm and ectoderm), and thus into all cells of the adult body. Multipotent and oligopotent stem cells, in order differentiate into fewer number of cell types. In embryonic development, there are two states of pluripotency, naive and primed pluripotency (Nichols and Smith, 2009). Pre-implantation blastocysts represent the naive state of pluripotency, whereas post-implantation blastocysts represent primed pluripotency. Naive and primed pluripotent cells represent different phases of development and are different in their morphology, X chromosome inactivation status, signaling requirements and gene expression (Nichols and Smith, 2009). Embryonic stem cells (ESCs) are pluripotent stem cells that are derived from the pre-implantation epiblast. These cells were first derived from murine embryos in 1981 (Evans and Kaufman, 1981, Martin, 1981) and seventeen years later from human embryos (Thomson et al., 1998). Pluripotent stem cells can also be artificially derived by inducing pluripotency in adult somatic cell types via ectopic expression of pluripotency factors. This process is called reprogramming and the pluripotent stem cells that are generated by this technology are referred to as induced pluripotent stem cells (iPSCs). Generation of iPSCs from mouse fibroblast was first reported in 2006 by Shinya Yamanaka’s laboratory (Takahashi and Yamanaka, 2006) and revolutionized the field of stem cell biology. Generation of iPSCs from human cells was reported one year later by two
independent groups, where two different sets of pluripotency factors were used for reprogramming; OCT4, SOX2, KLF4, C-MYC vs. OCT4, SOX2, Nanog and LIN28 (Takahashi et al., 2007, Yu et al., 2007). These studies made the derivation of patient-specific human iPSCs possible which today are being used for disease modeling and personalized drug discovery (reviewed in (Hockemeyer and Jaenisch, 2016)). Epiblast-derived stem cells (EpiSCs) are another type of pluripotent stem cells which are derived from post-implantation epiblasts and thus are classified as primed pluripotent stem cells. These cells were first derived in 2007 by Ludovic Vallier’s laboratory (Brons et al., 2007). The established pluripotent stem cell lines of each organism should reflect the characteristic features of their cell of origin. Therefore, to define these cellular and molecular features, it is critical to study the embryonic development of organisms independently.
2. Embryonic development

Embryonic development is the process during which the cellular progeny of a fertilized egg (zygote) divide, migrate and specialize. Embryonic development can be divided into two phases, namely pre-implantation and post-implantation embryonic development. Pre-implantation development initiates from the zygote and through several rounds of cell division results in the formation of a blastocyst with three distinct cell lineages: trophectoderm (TE), primitive endoderm (PE) and epiblast (EPI). Following implantation of a blastocyst into the uterus, TE and PE cell lineages give rise to extra-embryonic tissues, whereas the EPI cell lineage develops into the embryo proper. Upon implantation of the blastocyst, epiblasts cells undergo molecular and cellular changes and transit from naive to primed pluripotency. In placental mammals and upon implantation of the epiblast, one of two X chromosome in female epiblast cells becomes transcriptionally inactive. The silencing of the X chromosome is random and regulated by a long non-coding RNA (lncRNA), XIST, which itself is located on X chromosome (reviewed in (Minkovsky et al., 2012). The XIST-mediated silencing of the X chromosome in placental mammals is called X chromosome inactivation (XCI). XCI is a strategy to compensate for X-linked gene expression between male and female individuals.

Gene content and dosage compensation

Dosage compensation is a process whereby the dosage of X chromosome gene expression is adjusted both between the sexes and between the X chromosome and autosomes (Gupta et al., 2006, Nguyen and Disteche, 2006). Sex chromosome (X and Y) are significantly different in their gene content; the human X chromosome contains about 1100 genes, whereas the Y chromosome contains about 100 gene. This significant difference in gene content of sex chromosomes has led to evolution of dosage compensation mechanism concerning the dosage of gene products from XY and XX individuals, which was first described by Lyon in 1961 (Lyon, 1961). The presence of a second form of dosage compensation concerning the ratio of gene
products from a single X chromosome to diploid autosome sets was then revealed from studies performed on flies and several mammalian species. X:autosome gene expression balance was achieved in most tissues of males and females mammals by global upregulation of the single active X chromosome in order to roughly equalize with diploid autosomes (Nguyen and Disteche, 2006). Consistent with the concept of maintaining the balance of X:autosome gene expression, the upregulation of X-linked genes does not happen in haploid germ cells containing one set of autosomes.

There are different mechanisms to achieve dosage compensation between one copy of X chromosome in XY individuals, two copies of X chromosomes in XX individuals and two copies of autosomes (reviewed in (Disteche, 2012)). X dosage compensation is achieved through:

1. Upregulation of the single X in males to achieve dosage compensation between the sexes and between the X chromosome and autosomes; this form of dosage compensation is seen in Drosophila.

2. Transcriptional silencing of one of the two female X chromosomes (X chromosome inactivation; XCI) combined with upregulation of X-linked gene expression in both sexes; this form of dosage compensation is seen in mammals.

3. Partial transcriptional repression of both X chromosomes in XX females (X chromosome dampening; XCD) combined with upregulation of X-linked gene expression in both sexes; this form of dosage compensation is seen in Caenorhabditis elegans.

Of note, developmental timing to acquire dosage compensation varies among different organisms. Moreover, various organisms have developed diverse strategies to achieve X dosage compensation. For example, XCI can be achieved through imprinting or random inactivation. In imprinted forms of XCI, the paternal X chromosome (Xp) is chosen for inactivation; this form of XCI is seen in marsupials (non-placental mammal) and the initial round of XCI in pre-implantation mouse embryos. In random forms of XCI, one of the two X chromosome is randomly inactivated. Random XCI is regulated by upregulation of XIST from the future inactive X chromosome.

Dosage compensation is important in embryonic development and adult homeostasis (Schulz and Heard, 2013, Yang et al., 2016), and inappropriate X chromosome dosage compensation is associated with developmental abnormalities and cancer (reviewed in (Schulz and Heard, 2013)). Although haploinsufficiency on
a chromosome-wide scale in most organisms is not well tolerated, there are instances during embryonic development of some organisms where the X dosage compensation is absent. For example, mouse naive pluripotent epiblasts lack X dosage compensation between the sexes. It has been suggested that the lack of X dosage compensation at this developmental stage might be tolerated due to more rapid pre-implantation development in mice (Sahakyan and Plath, 2016). This phenomenon is also observed in mouse embryonic stem cells (mESCs) representing a similar X chromosome status to their embryonic cell or origin. However, despite the X chromosomal gene imbalance between the sexes of mESCs, the X to autosome gene expression seems to be adjusted by global DNA hypomethylation in XX female mESCs (Zvetkova et al., 2005). This suggests that some level of dosage compensation regulatory mechanism is present in mESCs.

Dosage compensation in mouse and human embryos

During mouse embryonic development, X dosage compensation occurs at two different developmental times (reviewed in (Chazaud and Yamanaka, 2016). It first occurs at the 4- to 8- cell stage through the imprinted form of XCI. This form of XCI is carried over to all cells of the morula; trophectoderm (TE) and the inner cell mass (ICM). Epiblast (EPI) and primitive endoderm (PE) cell lineages are specified from ICM at blastocyst stage, where the imprinted inactive X is specifically reactivated in epiblast cells. Thus, while the imprinted Xi is maintained in TE cells, the naive pluripotent EPI cells lack X dosage compensation between sexes and contain active X chromosomes (Xa) in both sexes (XaY males, XaXa females). Upon implantation of female embryo, EPI cells undergo random XCI regulated by Xist RNA and one of the two female X chromosome becomes inactive. Therefore, post-implantation primed pluripotent EPI cells possess X dosage compensation (XaY males, XX\textsuperscript{Xist+} females). The random Xi is carried over to all three cell lineages differentiated from post-implantation epiblasts and is maintained throughout the lifetime of the organism.

In human embryos, however, no imprinted XCI is observed, and the dosage compensation happens only at a later time and across all cells of blastocyst; TE, PE and EPI cells (Petropoulos et al., 2016). Moreover, the X dosage compensation between sexes is achieved through XCD (and not XCI) of female embryos, i.e. transcription of X-linked genes is equally lowered/dampened from two active X chromosomes of female pre-implantation blastocysts (Petropoulos et al., 2016).
Intriguingly, the XIST RNA is expressed from active X chromosomes of male and female pre-implantation embryo (Okamoto et al., 2011, Petropoulos et al., 2016). The level of XIST expression is much lower in male compared to female pre-implantation embryos (Petropoulos et al., 2016). This suggests that XIST expression in pre-implantation embryos is not coupled to X chromosome silencing. It remains unclear whether XCD is regulated by XIST expression. However, the negative correlation of gradual dampening of X-linked gene expression in female pre-implantation embryos and XIST upregulation from E4 to E7, and the lack of XCD in male X chromosomes, suggests the regulation of XCD by XIST expression (Sahakyan and Plath, 2016). Thus, female human pre-implantation epiblasts contain two XIST-expressing active X chromosomes with lowered X-linked gene expression and male human pre-implantation epiblasts contain one XIST-expressing active X chromosome without lowered X-linked gene expression (XaXIST+Y males, XaXIST+XaXIST+ females). Upon implantation, human epiblast cells undergo random XCI regulated by XIST expression similar to that seen in mouse embryonic development (XaY males, XiXIST+Xa females). The chromosome-wide random XCI that is acquired in mouse and human post-implantation embryonic development is maintained throughout the lifetime of these organisms.

Limitation of mouse studies for understanding human pre-implantation development

Mice have been the preferred mammals for studying pre-implantation embryonic development due to limited availability of human embryos. However, studies performed in mice cannot be directly applied to humans, due to significant differences between mouse and human pre-implantation development. Differences in developmental timing, gene expression, lineage segregation path, signaling requirements, dosage compensation processes and patterns of XIST expression are among the distinguishing features between human and mice pre-implantation development.

Lineage segregation path

In human embryos, TE- EPI- and PE- lineage segregation occurs simultaneously at the blastocyst stage (Petropoulos et al., 2016) rather than stepwise segregation as seen in mouse embryos, where TE and ICM are first segregated in the morula, and
then EPI and PE are specified from ICM at blastocyst stage (reviewed in (Chazaud and Yamanaka, 2016)).

Transcriptional state
Cells of the human epiblast prior to lineage segregation co-express lineage-specific markers of TE- EPI- and PE specific markers, implying a unique transcriptional plasticity in human epiblasts (Petropoulos et al., 2016).

Dosage compensation processes
Imprinted XCI does not occur in human pre-implantation embryos as seen in very early stages of mouse embryonic development. Moreover, the X chromosome dosage is compensated in late blastocyst stage of pre-implantation human embryos, whereas this mechanism is absent in mouse pre-implantation equivalents. The X dosage compensation in human pre-implantation embryos take place via XCD (Petropoulos et al., 2016) and not XCI. Thus, naive female pluripotent epiblasts in human embryos are characterized by two $XIST$ expressing active X chromosome with lowered/dampened X-linked gene expression ($X^aX^{IST+}$) (Petropoulos et al., 2016), whereas naive female pluripotent epiblasts in mouse embryos are characterized by two $Xist$ negative and active X chromosome ($XaXa$).

$XIST$ expression
In human pre-implantation embryos, $XIST$ is uniquely expressed from active X chromosome (Okamoto et al., 2011, Petropoulos et al., 2016) and thus is not coupled to X-linked gene silencing/inactivation. Instead, $XIST$ expression may be related to X-linked gene dampening/downregulation. In mice, $Xist$ expression is closely coupled to XCI.

$TSIX$ expression
The lncRNA $TSIX$ is not expressed in human embryos (Petropoulos et al., 2016) and thus cannot be involved in $XIST$ regulation. In contrast, $Tsix$ is detectable in mouse cells and represses $Xist$ expression via antisense regulation.
3. Pluripotent stem cells

PSCs are powerful research tool for studying early human development and modelling human diseases. In addition, the ability of PSCs to differentiate into all three germ layers make them a good candidate for use in potential therapeutic application. Thus, it is critical that these cells faithfully recapitulate the features of epiblasts and duplicate the exact transcriptional and epigenetic changes of in vivo differentiation; if not, the improper molecular features of these cells may interfere with biological interpretation or intended therapeutic applications of these cells.

States of pluripotency in mouse and human pluripotent stem cells

Mouse and human ESCs are derived from pre-implantation naive pluripotent epiblasts and thus should capture the molecular features corresponding to their embryonic cell of origin, i.e. naive pluripotency; mouse EpiSCs are derived from post-implantation epiblasts and should represent primed pluripotent epiblast cells. Epigenetic status of X chromosome of PSCs is a reliable molecular readout for evaluating developmental state of pluripotency (naive vs. primed). The epigenetic status of X chromosome in pre- and post-implantation epiblasts are referred to as pre- and post-XCI status, respectively. Post-XCI status in both mouse and human embryos is similar and is defined as XaXi$^{\text{XIST}+}$. However, pre-XCI state in mouse and human embryos is distinct and is defined as XaXa and Xa$^{\text{XIST}+}$Xa$^{\text{XIST}+}$, respectively.

Pluripotent stem cells

Mouse ESCs and iPSCs truly capture the pre-XCI status of mouse pre-implantation pluripotent epiblasts, XaXa, while human ESCs and iPSCs under conventional culture conditions do not recapitulate the pre-XCI status of naive pluripotent cells.
in human embryos. Human ESCs display either the prototypical post-XCI XaXi\textsuperscript{XIST}\textsuperscript{+} pattern or XaXa pattern (two active X without \textit{XIST} expression) (Figure 1). Likewise, human iPSC display the post-XCI XaXi\textsuperscript{XIST}\textsuperscript{+} pattern, but not the XaXa pattern. It has been proposed that the XaXa state is only specific to human ESCs and cannot be found in human iPSCs (Patel et al., 2017). It has been demonstrated that the XaXa state is stable and can be maintained overtime in culture (Patel et al., 2017); however, the XaXi\textsuperscript{XIST}\textsuperscript{+} state is unstable and can undergo erosion of X chromosome inactivation upon loss of \textit{XIST} expression from the inactive X chromosome (Shen et al., 2008, Silva et al., 2008, Mekhoubad et al., 2012, Nazor et al., 2012, Vallot et al., 2015, Patel et al., 2017) (Figure 1 and 2). Erosion of XCI results in partial reactivation of the inactive X chromosome (Xe: eroded Xi). The erosion of XCI can be moderate/slight (Xme: moderately eroded X) or vast/substantial (Xve: vastly eroded X) and is dependent on the number of X-linked genes that were affected (Bruck and Benvenisty, 2011, Patel et al., 2017, Moraghebi et al., Manuscript). However, the erosion does not result in complete reactivation of X chromosome; thus, the eroded X chromosome can be distinguished from the active X chromosome (Xa) (Patel et al., 2017). One example is the X-linked gene \textit{HUWE1}, which is subject to inactivation but is resistant to X reactivation. It can therefore be used as marker to distinguish between XaXa and XaXve. The epigenetic instability of the X chromosome impacts X-linked disease models by modifying the disease phenotype, thus highlighting the importance of monitoring the XCI status of the female lines during study of X-linked gene disease models (Mekhoubad et al., 2012).

Mouse PSCs, like their embryonic cell of origin, are capable of upregulating \textit{Xist} expression and inducing random XCI upon differentiation. On the other hand, human PSCs are not competent for \textit{XIST} upregulation and initiation of random XCI upon differentiation (Mekhoubad et al., 2012, Nazor et al., 2012, Patel et al., 2017). The X chromosome status of undifferentiated hPSCs is retained during differentiation and in differentiated cells. Thus, the improper X-linked genes expression in lines with XaXa/XaXe status is maintained in the differentiated cells (Mekhoubad et al., 2012, Nazor et al., 2012, Patel et al., 2017). It has been demonstrated that X-linked gene upregulation adversely affects the quality and differentiation propensity of the PSCs (Anguera et al., 2012, Bruck et al., 2013, Patel et al., 2017, Moraghebi et al., Manuscript). Thus, even though the XaXi\textsuperscript{XIST}\textsuperscript{+} hPSCs do not capture the correct X state of pre-implantation human embryos, it is the favorable phenotype for downstream application of hPSCs as X-linked genes are not inappropriately upregulated in these lines (Patel et al., 2017). However, the
ultimate goal is to identify the culture conditions that can establish hPSCs at the naive state of pluripotency.

Figure 1. Illustrating the X chromosome status of human pre-implantation epiblasts and embryonic stem cells (ESCs) derived under conventional culture conditions. The scheme summarizes changes that occur in the X chromosome status of female human ESCs during propagation and differentiation. Thick arrows represent full transcription from X chromosome and thin arrows represent dampened/lower transcription for X chromosome.
Of note, the X chromosome is enriched in genes related to sexual reproduction, brain function and cancer/testis antigen genes (Zechner et al., 2001, Khil et al., 2004, Vallender and Lahn, 2004, Ross et al., 2005, Simpson et al., 2005). GATA1 and ZIC3 are also encoded from X chromosome. GATA1 is required for erythroid and megakaryocyte maturation (Crispino, 2005, Ferreira et al., 2005) and its mutation in human causes congenital anemia and thrombocytopenia (Fujiwara et al., 1996, Campbell et al., 2013). ZIC3 is also required during early development for midline development and left-right asymmetry. ZIC3 also plays a role in maintaining pluripotency (Lim et al., 2007, Lim et al., 2010) and is a known regulator of Wnt/β-catenin signaling pathway (Fujimi et al., 2012, Diamand et al., 2017). There are numerous examples where improper expression of X-linked genes is associated with human disease. Thus, if human PSCs are to be used in embryonic development and disease modeling studies or regenerative medicine, it is critical that they can mimic the pre-XCI status of naive pluripotent epiblasts and X chromosome epigenetic changes of in vivo differentiation.
Figure 2. Illustrating the X chromosome status of human induced pluripotent stem cells (iPSCs) derived under conventional culture conditions. The scheme summarizes changes that occur in the X chromosome status of female human iPSCs during propagation and differentiation.

Epiblast stem cells

Mouse EpiSCs display the post-XCI status found in post-implantation embryos: XaXi^Xist+. These cells need FGF/Activin signaling for self-renewal, and differentiate
upon induction of Wnt/β-catenin signaling pathway. Likewise, human ESCs and iPSCs under conventional culture conditions display similar properties to mEpiSCs: They display post-XCI status, require FGF signaling for self-renewal and differentiate upon nuclear translocation of β-catenin and binding to T cell factor (TCF). This resemblance shows that hPSCs are developmentally closer to mEpiSCs and the primed state of pluripotency in post-implantation epiblasts (Nichols and Smith, 2009).

Towards generation of naive human pluripotent stem cells

True naive human female PSCs should resemble the X chromosome state of their embryonic cell of origin, i.e. two XIST-expressing active X chromosomes with XCD. Efforts to optimize culture conditions capable of converting the primed pluripotency state of conventional hPSCs to a pluripotency state that more closely resemble the naive pluripotency state in human embryos, has led to great achievements (Takashima et al., 2014, Theunissen et al., 2014). In these experiments, the X chromosome status has served as a readout to validate the efficacy of culture conditions. The resulting hPSCs obtained in the naive culture conditions are called naive hPSCs and show a more similar global gene expression profile to that of human pre-implantation embryos. These cells contain two active X chromosome (XaXa), express XIST RNA in a mono-allelic fashion (XaXa<sub>XIST+</sub>), and undergo non-random XCI upon differentiation; i.e. the prior Xi in conventional hPSCs undergoes XCI (Figure 3). During the conversion of primed to naive hPSCs, the epigenetic abnormalities of the X chromosome are erased; the XIST-expressing inactive X or eroded X chromosome become reactivated. Upon differentiation, the reactivated X chromosome upregulates XIST expression and becomes transcriptionally silent. Thus, differentiated daughter cells of naive hPSC do not display an inappropriate higher dosage of X-linked genes as observed in those differentiated from XaXa or XaXe conventional hPSCs.

Although, naive hPSC are much more similar to naive pluripotent cells of human embryos, they do not capture some of the molecular features of X chromosomes in human embryos, i.e. bi-allelic expression of XIST and random XCI upon differentiation. The non-random XCI of naive hPSCs implies the existence of epigenetic memory, and thus indicate the necessity of further modification to the current naive culture conditions or derivations in order to erase the remaining epigenetic memory and to achieve a developmental stage most similar to epiblasts of human pre-implantation embryos.
Figure 3. Illustrating the X chromosome status of human pre- and post- implantation embryos. The scheme also displays the X chromosome status of human female ESCs and iPSCs in both primed state of pluripotency and after transition to naive pluripotency. If the circle in the figure always represent the same X chromosome, it is obvious that in female human embryos both maternal and paternal X chromosome have the same probability of inactivation (random XCI). Whereas, naive pluripotent stem cells inactivate the prior inactive X chromosome in primed cultured condition (non-random XCI), suggesting an epigenetic memory from the inactive X chromosome of primed state.
Summary of XCI status of pluripotent cells *in vivo* and *in vitro*

**Mouse embryos**
- Pre-implantation naive pluripotent epiblasts: XaXa
- Post-implantation primed pluripotent epiblasts: XaX·Xist+

**Mouse PSCs**
- ESCs: XaXa
- iPSCs: XaXa
- EPiSCs: XaX·Xist+

**Human embryos**
- Pre-implantation naive pluripotent epiblasts: XaX·Xist+ XaX·Xist+
- Post-implantation primed pluripotent epiblasts: XaX·Xist+

**Human PSCs in conventional culture conditions**
- ESCs: XaXa or XaXiXIST+ or XaXe (XaXme or XaXve)
- iPSCs: XaXiXIST+ or XaXe (XaXme or XaXve)

**Human PSCs in naive culture conditions**
- ESCs: XaX·Xist+
- iPSCs: XaX·Xist+
4. Genetic and epigenetic variation in pluripotent stem cells

Many studies have reported genetic and epigenetic variations between different PSC lines, different passages of the same PSC line and between different populations at a specific passage of the same PSC line. These variations come from different sources; they may be inherited from the source cell population, introduced during reprogramming or accumulated during culturing. It has been demonstrated that some of these variations are relevant to the functionality of PSC cells and thus affect the utility and developmental potential of PSC lines. Thus, understanding the cause of genetic and epigenetic variation in hPSCs is of critical importance. Here, different types of genetic and epigenetic variation, their sources and the potential functional effects of these variations will be discussed.

Genetic variation

Different types of genetic variation may be detected in hPSCs: whole-chromosome variations (aneuploidy) or sub-chromosomal variations (CNV: copy number variation, SNV: single nucleotide variation). These genetic variations can be introduced into PSCs during their generation or maintenance. These variations can originate from the heterogeneous genetic makeup of source cell population or they can be culture-induced. The contribution of the reprogramming process to genetic variation in iPSC lines is controversial. The discord among reports arises from the sensitivity of the methods that were used to detect low frequency variations. While array-based studies suggested that the reprogramming process can result in genetic variation, sequencing-based studies have revealed many fewer or no detectable genetic variation caused by the reprogramming process. Thus, there is no solid support for the idea that genetic variation could be introduced during reprogramming, however this possibility cannot be excluded and needs further investigation.
Aneuploidy

Aneuploidy, or the presence of an abnormal number of chromosomes, can be detected by karyotyping and is frequently reported in in vitro cultured PSCs. The recurrent gains of specific chromosomes make up for more than half of the reported karyotype abnormalities (Amps et al., 2011, Taapken et al., 2011). Trisomy of chromosome 12, on which the pluripotency genes NANOG and GDF3 are encoded, is the most common reported aneuploidy in hPSCs (Draper et al., 2004, Mayshar et al., 2010, Amps et al., 2011, Taapken et al., 2011). A possible growth advantage conferred by the pluripotency factor NANOG could explain the frequent occurrence of trisomy 12 (Draper et al., 2004, Mayshar et al., 2010). Trisomy of chromosomes 8 and X are other examples of aneuploidy in hPSCs, which occur less frequently than trisomy 12 (Amps et al., 2011, Taapken et al., 2011). It has been demonstrated that aneuploidy in iPSC lines can be inherited from the source cells (Park et al., 2008); however, it is important to note that a high rate of aneuploidy in source cells does not necessarily translate into a similarly high rate of aneuploidy in the derived iPSC lines. It appears that reprogramming can select for or against the aneuploidy and that this is case dependent (Hamada et al., 2012). Long-term culturing of hPSCs is also associated with an increased frequency of aneuploidy (Amps et al., 2011, Taapken et al., 2011).

Sub-chromosomal variations

Copy number variation (CNV) is variation in smaller segments of the genome and can be detected by karyotyping or gene expression meta-analysis. Using a deep sequencing approach, it has been demonstrated that half of CNVs in hiPSCs are present in rare populations of their cell of origin and not introduced by the reprogramming process (Abyzov et al., 2012), challenging earlier studies using less sensitive detection methods that attributing detected CNVs in hiPSC lines to reprogramming (Hussein et al., 2011, Laurent et al., 2011). It appears that during in vitro culture of hPSCs, some CNVs can be selected for, including CNVs that occur at specific chromosomal locations such as the ones around pluripotency genes NANOG on chromosome 12 and DNMT3B on chromosome 20 (Mayshar et al., 2010, Laurent et al., 2011, Martins-Taylor et al., 2011). It has been also reported the CNV-originated genetic mosaicism that is observed between hiPSC lines at early passages can be lost upon in vitro culturing and passaging of the lines (Hussein et
al., 2011). However, using a deep sequencing approach, early passage mosaicisms could not be detected (Abyzov et al., 2012).

Single nucleotide variants (SNV) are differences in a single nucleotide that can be detected by high-throughput sequencing of the whole genome or exome. Most detected SNVs in iPSC lines are randomly distributed in the genome (Cheng et al., 2012, Ji et al., 2012, Young et al., 2012, Ruiz et al., 2013), and more than half of detected SNVs in exomes can be traced back to the source cells (Gore et al., 2011, Young et al., 2012).

Epigenetic variation

Different types of epigenetic variation may be detected in hPSCs: X chromosome-wide epigenetic variation and local epigenetic variation. Epigenetic variation, similar to the genetic variation, can be introduced into PSCs during their generation or maintenance. Likewise, they can originate from the source cell population or be induced by prolonged culturing. However, in contrast to genetic variation, reprogramming is one of the major contributors of epigenetic variation in hiPSC cells. Incomplete reprogramming or reprogramming-induced aberration in DNA methylation can lead to such variation. It has been demonstrated that epigenetic variations can increase the tumorigenic potential of hPSCs and alter their differentiation propensity.

Variations in epigenetic status of X chromosome

During reprogramming and under conventional culture conditions, female human somatic cells do not reactivate their inactive X chromosome and thus the XaXi status of parental cells is retained in hiPSCs (Tchieu et al., 2010, Pomp et al., 2011). Likewise, female human ESCs may also display XaXi state upon derivation under conventional culture conditions. PSCs with XaXi status are epigenetically unstable and have a tendency to undergo erosion of XCI (Mekhoubad et al., 2012, Patel et al., 2017, Sahakyan et al., 2017). The extent of this erosion varies between different lines and can result in reactivation of a varying number of X-linked genes, contributing to epigenetic variations between hPSC lines (Bruck and Benvenisty, 2011, Patel et al., 2017, Moraghebi et al., Manuscript). Human female epigenetic instability is attributed to culture conditions. Manipulation of culture conditions can
alter the epigenetic status of PSCs (Sahakyan et al., 2017). The X chromosome is enriched in cancer-related genes and upregulation of these genes can increase the risk of tumorigenicity of hPSCs. It has been demonstrated that erosion of XCI results in poor differentiation of hPSCs. Moreover, the eroded X chromosome dose not undergo XCI upon differentiation, and thus the inappropriate level of X-linked gene expression is transmitted to differentiated cells, altering their phenotype and possibly their properties (Mekhoubad et al., 2012, Nazor et al., 2012, Patel et al., 2017).

Variations in local epigenetic status

Source cell memory (epigenetic memory)
During reprogramming, the global epigenetic landscape of the source cell has to be reset in order to establish the epigenetic marks of the pluripotent state (reviewed in (Liang and Zhang, 2013a). This is achieved through DNA methylation and chromatin remodeling processes. However, resting of DNA methylation occurs at late stages of reprogramming and iPSC lines might be established before completion of this stage (Polo et al., 2012). And thus, hiPSC may retain some of the epigenetic signatures (DNA methylation pattern) of the source cell known as source cell, or epigenetic, memory (Kim et al., 2010, Polo et al., 2010, Bar-Nur et al., 2011, Kim et al., 2011, Lister et al., 2011, Ruiz et al., 2012). DNA hypomethylation of lineage-specific genes or DNA hypermethylation of genes that are required for specification of lineages other than the source cell lineage are examples of epigenetic memory. Of note, the somatic epigenetic memory can be erased over time by continued passaging; this needs to be considered when studying the effect of epigenetic memory on hiPSC functionality. It has been reported that the differentiation potential of iPSC lines is skewed in favor of their source cell lineage (Kim et al., 2010, Polo et al., 2010, Bar-Nur et al., 2011, Kim et al., 2011). However, other studies have demonstrated that epigenetic memory is not the main driver functional differences in hiPSC lines (Kajiwara et al., 2012, Rouhani et al., 2014, Kyttala et al., 2016).

Aberrant DNA methylation
Aberrant DNA methylation patterns may be induced during reprogramming, which is distinct from those originating from source cells. Reprogramming-induced aberrant DNA methylation has been reported at certain imprinted loci (Pick et al., 2009, Chamberlain et al., 2010, Nishino et al., 2011, Nazor et al., 2012). Aberrant
methylation of imprinted locus H19 was also reported upon prolonged passaging of hPSCs (Nishino et al., 2011, Nazor et al., 2012). Reprograming-induced aberration in DNA methylation is not specific to imprinted loci and can be also detected in other genomic regions (Lister et al., 2011, Ruiz et al., 2012). Taken together, aberrant DNA methylation can be induced by reprogramming or prolonged culture conditions.
5. Hematopoiesis

Hematopoiesis is the process by which the new blood cells are formed. Maintenance of adult hematopoiesis relies on self-renewing hematopoietic stem cells (HSCs) that reside in the bone marrow (BM) and give rise to all the different mature blood cell types. Although BM is the main site of hematopoiesis in the adult, HSCs are generated during embryonic development and home to the bone marrow pre-natally. It appears that several diseases of the hematopoietic system arise in early human development and in utero (Hunger et al., 1998, Greaves, 2005, Hong et al., 2008, Roy et al., 2012, Barrett et al., 2016). This makes understanding of embryonic hematopoiesis very important.

Developmental hematopoiesis in mammals

In mammals, embryonic hematopoiesis consists of three programs: primitive, definitive and transient definitive (also known as secondary primitive) which occur at different times and anatomical sites during embryonic development. These forms of hematopoiesis are different in developmental potential, i.e. only definitive hematopoiesis can generate HSCs, which are defined by their long-term repopulation capacity and lymphoid potential (reviewed in (Dzierzak and Speck, 2008)). Transient definitive hematopoiesis is also capable of generating definitive lymphocytes (Boiers et al., 2013) but cannot generate HSCs. However, it is important to note that the ability of multi-lineage long-term reconstitution is not specific to HSCs and that immature HSCs (pre-HSC) also capable of reconstituting mice and NK engraftment (Rybtsov et al., 2011, Kiesusseian et al., 2012).

Primitive hematopoiesis features the formation of first hematopoietic cells in the extra-embryonic tissue yolk sac (Silver and Palis, 1997). This form of hematopoiesis is transitory and represents an HSC-independent hematopoietic program which is required for embryonic development. Transient definitive (secondary primitive) hematopoiesis initiates a bit later in the yolk sac and gives rise to erythro-myeloid progenitors (EMP) and lymphoid progenitors (McGrath et al., 2015) that transiently
seed the fetal liver. Definitive hematopoiesis initiates later in the aorta-gonad-mesonephros (AGM) region of the embryo proper and is characterized by the emergence of HSCs that are capable of multi-lineage long-term reconstitution. Definitive hematopoiesis gives rise to the permanent adult hematopoietic system (Medvinsky et al., 1993, Medvinsky and Dzierzak, 1996).

Besides functional HSCs, adaptive B-2 lymphocytes can also indicate definitive hematopoiesis as these cells arise exclusively from HSCs. During mouse embryonic development, B lymphocytes are produced in three waves (Herzenberg and Herzenberg, 1989). The first wave of B cell production occurs in yolk sac and AGM before HSC emergence. The B cells produced are the innate type B-1 lymphocytes which arise independently of HSCs and seed the fetal liver (Yoshimoto, 2015). The second wave occurs in the fetal liver and consists of innate B-1 and adaptive B-2 lymphocytes (Yoshimoto, 2015). These cells are differentiated from HSCs and thus HSC dependent. The last wave of B cell production during mouse embryonic hematopoiesis occurs in the bone marrow and mainly produces adaptive B-2 cells from HSCs (Montecino-Rodriguez and Dorshkind, 2012). As adaptive B-2 lymphocytes can only be generated from HSCs and that they can be distinguished from B-1 immunophenotypically, B-2 cell production can be an indicator of definitive hematopoiesis. Innate B-1 lymphocytes have also been identified in human development, demonstrating the existence of the innate immune system in human embryos (Rothstein and Quach, 2015, Bueno et al., 2016).

**Origin of the hematopoietic cells**

In 1932, it was suggested that hematopoietic and endothelial cells in blood island of chick embryo are closely related through a common mesodermal precursor, termed the hemangioblast (Murray, 1932). Isolation of cells from the primitive streak at mouse E7.5 and generation of precursor cells from mouse ESCs with similar bi-potential potential has further confirmed that endothelial cells and hematopoietic cells share a common precursor (Kennedy et al., 1997, Choi et al., 1998). *In vitro* ESC differentiation experiments further described that the development of hematopoietic cells proceeds sequentially from mesoderm through the hemangioblast to hemogenic endothelium to hematopoietic progenitors (Eilken et al., 2009, Lancrin et al., 2009). Hemogenic endothelium (HE) is a term used for describing a specialized subset of endothelial cells with hematopoietic potential. Indeed, hematopoietic cells have been shown to emerge from endothelial cells of
aorta (Garcia-Porrero et al., 1995, Tavian et al., 1996, Jaffredo et al., 1998, Boisset et al., 2010), and has been additionally verified by in vivo lineage tracing experiments (Zovein et al., 2008, Rhodes et al., 2008, Chen et al., 2009, Bertrand et al., 2010, Kissa and Herbomel, 2010).

It has been demonstrated that Runx1 is required for endothelial to hematopoietic transition (EHT) and intra-aortic hematopoietic cluster (IAHC) formation; Runx1-deficient mice lack definitive hematopoiesis (Okuda et al., 1996, North et al., 1999, Cai et al., 2000, Chen et al., 2009). This confirms that definitive hematopoiesis transit through HE. In contrast, primitive hematopoiesis in the yolk sac of mouse embryos is Runx1-independent; mice with homozygous mutation in Runx1 showed normal yolk sac-derived erythropoiesis but lacked fetal liver hematopoiesis, suggesting that mouse primitive hematopoiesis is directly derived from mesoderm and does not transition through HE (Okuda et al., 1996, Ueno and Weissman, 2006). However, in vitro human ESC differentiation proposes that both primitive and definitive hematopoiesis transit through HE (Sturgeon et al., 2014).

A recent study has demonstrated that the specification of hematopoietic and endothelial cells in the yolk sac of mouse embryo does not involve an intermediate hemangioblast, challenging the existence of common precursor hypothesis. Instead they have suggested that the development of definitive hematopoietic cells proceeds sequentially from mesoderm through the Runx1+/CD41+ hemogenic endothelium to hematopoietic progenitors (Padron-Barthe et al., 2014).

Developmental hematopoiesis in human

During human embryonic development, the first wave of primitive hematopoiesis occurs in the yolk sac at CS7-8 (CS: Carnegie stage) and mainly creates large nucleated erythrocytes and occasionally CD45+ monocytes/macrophages and megakaryocytes (William Bloom and Bartelmez, July 1940, Fukuda, 1973, Luckett, 1978). These cells provide functional hematopoietic cells required for embryo growth and tissue differentiation. Primitive hematopoietic cells can be detected in the fetal liver at CS 10; the onset of blood circulation (Tavian et al., 1999). There is evidence for a possible second transient wave of yolk sac-derived hematopoiesis giving rise to erythro-myeloid progenitors (Migliaccio et al., 1986), (EMP) similar to that seen in mouse (Boiers et al., 2013, McGrath et al., 2015).
At CS13 the endothelial cells lining the dorsal aorta give rise to hematopoietic progenitor cells and gradually replace primitive erythrocytes and CD45+ cells in fetal liver through bloodstream (Tavian et al., 1999). HSCs emerge in the AGM region at CS14 and persist until CS17, which marks the onset of the permanent and definitive wave of adult hematopoiesis (Tavian et al., 1996, Tavian et al., 1999, Tavian et al., 2001, Oberlin et al., 2002). Both hematopoietic stem and progenitor cells are organized within intra-aortic hematopoietic clusters (IAHCs) at the floor of dorsal aorta where the pre-umbilical region of dorsal aorta is located (Tavian et al., 1996, Tavian et al., 1999). Xenotransplantation experiments has shown that during human embryonic development, AGM generates only one or very few definitive HSCs. (Ivanovs et al., 2011). AGM-derived HSC possess an exceptional self-renewal potential with an average replication rate of at least 3.5-4 times per weeks (Ivanovs et al., 2011).

HSCs appear in the yolk sac and fetal liver at CS16 and CS17, respectively. CD34+ cells with hematopoietic potential are found in placenta from week 5-6 of gestation (Muench et al., 2017), where terminal maturation and enucleation of primitive erythrocytes occurs (Van Handel et al., 2010). In placenta, transplantable HSCs have been detected by 6 weeks of gestation (Robin et al., 2009). However other studies have suggested that HSCs cannot be detected until after week 9 of gestation or the second trimester (Ivanovs et al., 2011, Muench et al., 2017). It has been demonstrated that human fetal HSCs with self-renewal ability express GPI-80; the GPI-80+ HSPCs appears in placenta by 5 weeks of gestation (Prashad et al., 2015).

After the emergence of HSC in AGM, these cells travel through bloodstream and colonize the fetal liver where they undergo massive expansion. Following the expansion phase in the fetal liver, the fetal BM takes over the hematopoietic activity by first being seeded by myeloid progenitors at 10.5 week after gestation and later by HSCs after 16 weeks of gestation. The migration of HSCs into BM is associated with invasion of blood vessels into cartilaginous bone, chondrolysis and bone ossification (Charbord et al., 1996). HSCs then reside in the BM during the entire life of an individual.

**Functional assays for studying human hematopoiesis**

Multiple methods have been developed to study human hematopoietic development. In 1999, the colony forming unit-cell (CFU-C) assay was developed to assess the presence of functional hematopoietic progenitors (Tavian et al., 1999). In this assay,
the ability of hematopoietic progenitors to proliferate and differentiate into hematopoietic colonies of different lineages is measured in response to hematopoietic cytokine stimulation in a semi-solid media. CFU-Cs are counted and characterized based on their morphology. This assay is unable to evaluate the potential of embryonic tissues to generate hematopoietic cells. In 1996, explant culture of mouse embryonic tissue was developed and helped to resolve this issue (Cumano et al., 1996, Medvinsky and Dzierzak, 1996). In this assay, further development of embryonic tissues is allowed through explant cultures in vitro and before assessing hematopoietic potential. In 2001, this assay was successfully adopted in human embryonic studies and could distinguish between the hematopoietic differentiation potential of yolk sac and AGM (Tavian et al., 2001, Oberlin et al., 2002). However, none of these assays truly examine both self-renewal and multi-potentiality of HSCs. The only way to truly evaluate the functionality of HSCs is to test their ability to serially repopulate myeloablated recipients. The xenotransplantation of human cells into mice first became possible in 1988, when severe combined immune-deficient (SCID) mice were generated; however, first generation SCID mice often rejected xenografts (McCune et al., 1988). The introduction of the humanized mouse model NOD/SCID IL2Rγnull (non-obese diabetic-severe combined immune deficiency, NSG) mice has led to an important improvement of in vivo xenotransplantation assay to study human hematopoiesis. These mice lack functional B, T, antigen presenting and NK cells (Shultz et al., 1995, Shultz et al., 2005).
6. Modelling and studying human developmental hematopoiesis

As mentioned earlier, there are various hematopoietic diseases that arise pre-natally and thus it is important to study hematopoiesis during embryonic development. Today, different strategies are used for studying human developmental hematopoiesis including the use of mouse models, in vitro differentiation of human pluripotent stem cells and teratoma formation. The extent to which these models recapitulate human developmental hematopoiesis are discussed below.

**In vivo models**

**The mouse as a model**

Over the years, mouse models have been widely used for studying developmental hematopoiesis given the ease of genetics and embryology. Much of our understanding regarding developmental hematopoiesis comes from decades of studies performed in mice. The mouse has been an invaluable tool for defining the onset of hematopoiesis, various anatomical sites of hematopoiesis, different hematopoietic programs and the key regulators of hematopoietic development (reviewed in (Sykes and Scadden, 2013, Ivanovs et al., 2017)).

Although the studies of mice are very powerful and share commonalities with human, the use of mouse models for human developmental hematopoiesis has some limitations due to species-specific differences (reviewed in (Sykes and Scadden, 2013, Ivanovs et al., 2017)). The differences in gestational period, developmental timescale, replicative rate of stem cells, differential cytokines requirements for HSC development (Sitnicka et al., 2003), differential yolk sac structure and location of IAHC emergence in dorsal aorta are among these differences. These variations can complicate the interpretation of data obtained in mice and thus urge caution when
extending these results to humans. This highlights that mice should only be used as a guide and that the data obtained must be verified in humans.

**Teratoma formation as a model**

Teratoma formation has been used as an *in vivo* human PSC differentiation system. Human PSCs can be injected into immunodeficient mice, where they can engraft and differentiate into derivatives of all three germ layers. Two independent studies have shown that human CD45+ can be generated in teratomas (Amabile et al., 2013, Suzuki et al., 2013). These PSC-derived CD34+CD45+ cells can then be isolated from teratomas and transplanted into a secondary immunodeficient mouse. The transplantation of these cells supported multilineage reconstitution of secondary mice including human CD235a+ erythrocytes and human CD3+ T cells. These experiments were performed with the ultimate goal of *in vivo* generation of transplantable HLA-matched HSCs. Today, HSCs can be also generated *in vitro* from patient derived iPSCs. The hematopoietic cells that are generated by teratoma formation, can also be used for drug screening applications.

**In vitro model**

**Human pluripotent stem cell as a model**

To validate the data obtained from mouse studies in human, primary human fetal tissue obtained from spontaneous abortions or elective termination of pregnancy can be used. However, availability of these tissues is often challenging due to ethical concerns, and thus establishing cell lines that are capable of mimicking embryonic hematopoiesis is of great interest. There is convincing evidence that human PSC differentiation can recapitulate the key stages of embryonic hematopoiesis. Here, different aspects of hematopoietic development that can or cannot be recapitulated by hPSCs will be discussed.

The establishment of the embryonic hematopoiesis system involves multiple developmental steps ranging from mesoderm induction and patterning to primitive streak and hematopoietic cell specification. This is achieved by the concerted actions of many signaling pathways such as FGF, BMP4, TGF-β, WNT, RA and Notch (Lawson et al., 2001, Gering and Patient, 2005, Chanda et al., 2013, Lizama
et al., 2015, Ciau-Uitz et al., 2016). Studies of human PSC have demonstrated that mesoderm induction and patterning as well as primitive streak specification can be recapitulated through the combined activities of the FGF, BMP4, canonical WNT, and activin signaling pathways (Schier and Shen, 2000, Kimelman, 2006, Nostro et al., 2008, Woll et al., 2008, Wang and Nakayama, 2009, Bernardo et al., 2011, Yu et al., 2011). Mesoderm cells committed to hematopoietic differentiation express the primitive streak transcription factors T (brachuary), MIXL1, FOXF1, and cell surface receptors KDR and PDGFRA (Davis et al., 2008, Slukvin, 2016, Ditadi et al., 2017).

Studies with hPSCs have shown that BMP4 signaling induces mesoderm differentiation and a combination of BMP4 and activin signaling promote a primitive hematopoietic program similar to that in the yolk sac (Nostro et al., 2008). The BMP4-induced mesoderm after 2 to 4 days of differentiation gives rise to CD235a-expressing blast-colony forming cells (BL-CFCs) (Kennedy et al., 2007, Davis et al., 2008, Vodyanik et al., 2010), which in turn give rise to primitive erythrocytes, megakaryocytes and macrophages (Lancrin et al., 2009). CD235a expression at the early stages of hPSC differentiation marks the primitive hematopoietic program (Sturgeon et al., 2014). The hematopoietic cells generated with such a differentiation protocol are erythroid-biased and the erythroid cells generated with this protocol express embryonic globin similar to that of the yolk sac primitive hematopoiesis (Chang et al., 2006, Lu et al., 2008, Hatzistavrou et al., 2009, Dias et al., 2011). This culture can be further differentiated using OP9 stromal cell co-culture (Vodyanik et al., 2006, Pick et al., 2007, Nostro et al., 2008, Uenishi et al., 2014), which results in sequential generation of CD34+ endothelial cells, CD34+CD43+ hematopoietic progenitor cells, and myeloid and erythroid cell lineages (Choi et al., 2012, Kennedy et al., 2012, Ng et al., 2016). The hematopoietic cells generated from this culture are not erythroid-biased and have clonogenic potential. The erythrocytes generated from these prolonged cultures mainly express fetal globin, and sometimes adult globin expression is also observed in such cultures (Qiu et al., 2008, Lapillonne et al., 2010, Yang et al., 2014, Fujita et al., 2016). During human embryonic hematopoiesis, EMPs with similar properties were first detected in the yolk sac and later in AGM. However, there is one known distinguishing feature between the hematopoietic cells derived from yolk sac and other sites of embryonic hematopoiesis: selective expression of HOXA genes. Yolk sac-derived hematopoietic cells do not express HOXA genes, whereas hematopoietic cells isolated from AGM, fetal liver and umbilical cord blood (UCB) express HOXA genes. The PSC-derived hematopoietic progenitors do not express HOXA genes.
(Dou et al., 2016, Ng et al., 2016) and thus likely represent the equivalent to the yolk sac EMPs.

Other studies have demonstrated that the erythroid-biased primitive program can be inhibited by initial culture of differentiating hPSC with BMP4 followed by a brief inhibition of activin signaling (Kennedy et al., 2012) or activation of WNT signaling (Gertow et al., 2013, Sturgeon et al., 2014) or a combination of both (Ng et al., 2016). It has been demonstrated that inhibition of activin signaling alone results in an increase in the expression of HOXA3, HOXA7 and HOXA9, and activation of WNT signaling alone results in increased expression of HOXA9 and HOXA10, whereas a combination of both results in an even higher upregulation of HOXA3, HOXA5, HOXA7, HOXA9 and HOXA10 (Ng et al., 2016). These culture conditions have been shown to support “definitive” hematopoiesis. The hematopoietic cells generated with these differentiation protocols were considered to be part of a definitive hematopoiesis program due to the expression of fetal globins and T cell differentiation potential in the presence of Notch signaling (Kennedy et al., 2012, Sturgeon et al., 2014). However, the generation of T lymphocytes is not exclusive to AGM-derived HSCs and studies in mice have provided evidence that B and T lymphocytes can be generated in the yolk sac and before HSC emergence (Yoshimoto et al., 2011, Yoshimoto et al., 2012, McGrath et al., 2015). The hematopoietic cells generated with these protocols are not capable of multi-lineage long-term reconstitution upon transplantation but express HOXA genes and thus are likely equivalent to AGM-derive EMPs.

Finally, a recent study has demonstrated that human PSC-derived hemogenic endothelium can be converted to multi-lineage long-term repopulation HSCs by overexpression of seven transcription factors ERG, HOXA5, HOXA9, HOXA10, LCOR, RUNX1 and SPI1 (Sugimura et al., 2017). This study will help us to better understand the transition for HE to hematopoietic cells, and the next step would be to generate the functional HSCs by inducing the expression of these seven transcription factors using morphogens instead of transgenes.
7. General Discussion and future perspectives

There is significant genetic and epigenetic heterogeneity among hPSC cultures. Some of these variations have no impact on the properties of hPSCs and thus can be disregarded. However, other variations affect the utility and functionality of hPSCs, and thus need to be addressed. Erosion of XCI in human female PSCs, which results in inappropriate X-linked gene expression, is such an example and has been shown to correlate with accelerated growth rate and poor differentiation potential (Anguera et al., 2012, Patel et al., 2017, Moraghebi et al., Manuscript). Differences in the expression of the X-linked gene ELK-1 correlates with programmed cell death and differentiation potential (Bruck et al., 2013). Upon differentiation, this improper X-linked gene expression is transmitted into the differentiated cells and alters their phenotype and possibly their properties (Mekhoubad et al., 2012, Nazor et al., 2012, Patel et al., 2017). In addition, it has been demonstrated that the level of IGF2 expression correlates with hematopoietic commitment capacity, and that the reprogramming-induced aberrant DNA methylation affect hematopoietic maturation and hepatic differentiation (Nishizawa et al., 2016). All these studies demonstrate that genetic and epigenetic variations can affect hPSC downstream application and urge us to avoid using hPSCs with genetic and epigenetic variation that can compromise their utility and functionality. While basic analyses such as karyotyping, expression analysis of core pluripotency genes and teratoma formation are essential to characterize hPSCs, more comprehensive genome-wide or locus-specific genetic and epigenetic analyses are required in order to detect the genetic and epigenetic variations throughout passages.

Different strategies have been proposed to reduce the probability of occurrence of these variations (reviewed in (Liang and Zhang, 2013b). Using young cells with minimal exposure to mutagens and selecting cells with the fewest epigenetic abnormalities for iPSC generation have been proposed to reduce variations originating from donor cells. In addition, using non-integrating vectors, changing the stoichiometry of reprogramming factors and using chemical inhibitors of DNA
methylation and histone deacetylation have been suggested as solutions to reduce variations that are induced or selected for by the reprogramming process. Likewise, using defined media and optimized culture conditions capable of preserving genetic and epigenetic integrity can help with reducing the variations that are induced by culture. In addition, it is important to consider the passage number of hPSCs when using them for different applications. It has been suggested that passaging diminishes the somatic cell memory (Nishino et al., 2011), but at the same time, certain genetic and epigenetic variations can be induced or accumulated throughout passages.

Some genetic and epigenetic variation such as aneuploidy or erosion of XCI in hPSCs are artefactual, whereas other variations correspond to different developmental states of pluripotency; i.e. naive vs. primed pluripotency. Variations within PSC lines can be indicators of distinct lineage-specific progenitors that are present in subpopulations of the same PSCs, e.g. Wnt high and Wnt low hPSCs with distinct developmental potential can coexist within in the same PSC line. The level of Wnt activity in hPSCs correlated with differences in the expression of lineage-specific markers and in the differentiation propensity of the cells (Blauwkamp et al., 2012). These types of variation are persistent, i.e. clonally isolated subpopulation rapidly reestablish the other populations to generate an equilibrium among different subpopulation (Blauwkamp et al., 2012). This suggest that the heterogeneity among hPSCs is one of the major cause of inefficiency in directing hPSCs towards specific cell lineages. It has been demonstrated that manipulating culture conditions can reduce this heterogeneity and lead to a more efficient derivation of targeted cell type from hPSCs. For example, stimulating Wnt signaling in hPSC cultures results in a Wnt high homogeneity which can differentiate towards mesendoderm cell lineages with a greater efficiency compared to heterogenous cell population (Blauwkamp et al., 2012). Likewise, Wnt inhibition in hPSC cultures results in a Wnt low homogenous cell population which differentiate towards mesendoderm cell lineages with an enhanced ability compared to heterogenous population.

Experiments with hPSCs show that it is possible to model hematopoietic development in vitro as their differentiation recapitulates key stages of embryonic hematopoiesis. However embryonic hematopoiesis consists of primitive and definitive programs with different signaling requirements, and thus different differentiation strategies are required for specification of each program. Both canonical Wnt and Nodal signaling are required for primitive streak specification from hPSCs. It has been demonstrated that direct interactions between β-catenin and SMAD2/SMAD3 in proximity to OCT4 binding in upstream regulatory regions of
primitive streak genes are required to specifically activate primitive streak genes in hPSCs (Funa et al., 2015). Experiments with human PSCs have suggested that activation of Wnt signaling during early mesoderm specification inhibits primitive hematopoiesis (Gertow et al., 2013, Sturgeon et al., 2014). BMP4 induced mesoderm differentiation and a combination of BMP4 and activin signaling seems to be sufficient to specify primitive program from hPSCs (Nostro et al., 2008). Studies with hPSCs have demonstrated that Wnt/β-catenin signaling is required for specification of definitive hematopoiesis from hPSCs (Gertow et al., 2013, Sturgeon et al., 2014, Ng et al., 2016), establishing a role for Wnt signaling at primitive streak and early mesoderm specification stage of definitive hematopoietic development. These experiments show that sufficient level of endogenous Wnt signaling in hPSCs is required in order to establish the definitive hematopoietic fate. In fact, the level of Wnt3 in hPSCs is used a predictive marker of definitive endoderm differentiation (Jiang et al., 2013). Wnt signaling regulates hematopoiesis in a dosage and context dependent manner. In fact, high doses of Wnt signaling may compromises erythropoiesis (Paluru et al., 2014), which highlights the importance of establishing the appropriate dosage of Wnt signaling for each step definitive hematopoietic development. Given the significant variability of endogenous Wnt signaling between different hPSC lines, the appropriate level of Wnt signaling for each line might be different.

Despite the great achievements in establishing different differentiation strategies for generation of either primitive or definitive hematopoietic program, where many aspects of embryonic hematopoiesis are accurately recapitulated in vitro by differentiating hPSCs, these differentiation protocol cannot generate transplantable HSCs without using transgenes or efficiently produce B cells (reviewed in (Ivanovs et al., 2017)).

To further improve the differentiation protocol, we need to better understand the embryonic hematopoiesis and key determinants of HSC fate. Most of the genes that are used for programing or reprogramming towards HSCs come from the differential gene expression analysis of hematopoietic stem and progenitor cells isolated from bone marrow, fetal liver and umbilical cord blood. In order to be able to generate genetically unmanipulated HSCs in vitro, we need to better understand the developing HSC hierarchy and identify cells upstream of pre-HSCs. The next step would be to isolate the cells upstream of pre-HSCs and compare their gene expression analysis to HSCs isolated from AGM.
In summary, in order to use hPSCs to model embryonic hematopoiesis, it is important to make an effort to reduce the variations that are inherited or induced/selected during the reprogramming process or throughout passages and adversely affect hematopoietic differentiation or maturation potential of hPSCs. It is also critical to establish hematopoietic differentiation protocol that can faithfully recapitulate the exact transcriptional and epigenetic changes of embryonic hematopoietic differentiation. This will be only possible by better understanding the mechanism of reprogramming to pluripotency and embryonic hematopoietic development.
8. Summary of articles

Article I

*Genetic variability overrides the impact of parental cell type and determines iPSC differentiation potential.*

In article I, we evaluate the contribution of donor- and tissue-specific genetic and epigenetic variations to hiPSC heterogeneity and differentiation potential. We demonstrate that donor-specific genetic and epigenetic variations are the main causes of hiPSC variability and differences in their differentiation potential.

We derived iPSC lines from different donors and different cell types (fibroblast and blood cells) of the same donor and performed gene expression and methylation analyses on these lines. Unsupervised hierarchical clustering of iPSC lines based on their gene expression or DNA methylation profile did not group them according to the cell type of origin, but rather in a donor-dependent manner. Moreover, we demonstrated that differentially expressed genes in iPSCs of different donors are maintained throughout spontaneous differentiation and are transferred to embryoid bodies. In vitro analysis of the hematopoietic lineage potential of the iPSC lines showed a significant difference in differentiation potential of iPSCs derived from different donor and not based on cell type of origin. Taken together, we showed that the variability in iPSCs resulting from genetic and epigenetic differences in normal donors resulted in far greater variability in transcriptional profile and epigenetic marks, and more importantly in functional differentiation potential of the pluripotent stem cells compared to the variability introduced from using differing source cells.
Article II

*Term amniotic fluid: an unexploited reserve of mesenchymal stromal cells for reprogramming and potential cell therapy applications.*

In article II, we evaluated term amniotic fluid derived MSCs (TAF-MSCs) as starting cell material for reprogramming to pluripotency and confirmed the ability of these derived iPSCs (TAF-iPSCs) to differentiate towards blood and neural cell lineages. When selecting source cells for reprogramming, using cells from embryonic, neonatal or juvenile tissues with the lowest accumulation of genetic mutations is recommended. TAF-MSCs are from a neonatal source and therefore have advantages compared to adult somatic cells, such as reduced acquired mutations and greater proliferative potential.

By using a novel collection system, we demonstrated that retrieval of significant volumes of term amniotic fluid and its cellular constituents in connection with planned caesarean section deliveries is feasible. The broad potential donor base of TAF-MSCs (approximately 3 million caesarean section deliveries per year in OECD countries), and their neonatal origin, support the idea of establishing an iPS bank with broad spectrum HLA types and genetic backgrounds. With the results from article I showing a rather large effect of genetic background on hematopoietic differentiation potential of hiPSC lines, we propose that each line in the hiPSC bank should be profiled for its genetic and epigenetic signature and examined for differentiation potential towards different cell lineages.

Article III

*Epigenetic variation of X chromosome inactivation in female human pluripotent stem cells is correlated with differentiation propensity.*

In article III, we evaluated the effect of epigenetic variation on hematopoietic differentiation potential of isogenic human female iPSCs and demonstrated that erosion of XCI is adversely correlated with hematopoietic differentiation potential of these lines. Our finding fits with the field’s current understanding of epigenetic instability of female hPSCs passaged under conventional culture conditions,
whereby the erosion of XCI has been negatively correlated with differentiation potential.

We identified 9 candidate X-linked genes including ZIC3 that could be correlated with the hematopoietic differentiation defects seen in lines with extensive erosion of XCI. We showed that inappropriate expression of the X-linked gene ZIC3 in vastly eroded iPSC lines had a negative impact on their hematopoietic differentiation capacity. ZIC3 is a known regulator of Wnt signaling, which is essential for definitive hematopoietic development. We demonstrated that the low hematopoietic differentiation capacity of vastly eroded lines can be rescued by chemical induction of Wnt signaling, and thus suggest a role for ZIC3-mediated Wnt signaling in hematopoietic differentiation of hPSCs. These results reinforce the effect of epigenetic variation on hPSCs functionality and highlights the importance of evaluating XCI status in female hiPSCs cultured under primed conditions.
Populärvetenskaplig sammanfattning på svenska

Embryonala stamceller är odifferentierade (omogna) celler med kapacitet att bilda alla typer av celler i kroppen. Dessa celler hittas i embryonal vävnad och förser kontinuerligt embryot med nya celler. En motsvarighet till dessa naturligt förekommande embryonala stamceller kan framställas i laboratoriet genom att manipulera differentierade celler, dvs redan specialiserade (mogna/färdigutvecklade) celler, och få dem att bli odifferentierade. Celler som framställs på detta sätt kallas inducerade pluripotenta stamceller (iPSC) och de uppvisar samma egenskaper som embryonala stamceller; de kan utvecklas till att bli nästan alla typer av specialiserade celler. Humana iPSC används idag i biomedicinsk forskning och för utveckling av cellterapi. Cellerna kan t ex härma embryots utvecklingsfaser och tack var detta kan man studera embryonal utveckling. iPSC kan också framställas från sjuka celler och man kan på så sätt studera humana sjukdomar i cellodling. Humana iPSC har också bl a förmågan att differentiera till blodceller och kan på så sätt i framtiden vara en källa till artificiellt blod för behandling av flertalet blodsjukdomar. iPSC kan framställas från patienters kroppsegnadceller och tack vare detta kommer iPSC-deriverade blodceller vara HLA-matchade och kommer således inte stötas bort av patientens immunförsvar vid transfusion eller transplantation.

Humana iPSC är ofta olika i sina genom (dvs den nedärvda genetiska informationen i DNAt) och cellerna skiljer sig även i de funktionella produkter som bildas från genomet (genexpression). Dessa skillnader kan ha ursprung i olika källor. De kan nedärvas från de specialiserade donatorceller som använts för att tillverka iPSC-cellerna, men kan också uppkomma under konversionen från specialiserad donatorcell till icke-differentierad iPSC-cell. Dessutom kan de uppstå under odling av iPSC-cellerna i laboratoriet. Vissa av skillnaderna som uppstår kan ha en negativ påverkan på iPSC-cellernas förmåga att differentiera till diverse specialiserade celler, däribland blodceller. Exempelvis kan låga nivåer av en genprodukt vid namn IGF2 minska iPSC-cellers förmåga att bilda blodceller. Av denna anledning är det viktigt
att studera och identifiera vilka faktorer som kan ha en negativ påverkan på iPSC-cellarers förmåga att bilda olika typer av celler.

Vissa forskare påstår att iPSC har kvar ett minne den ursprungliga specialiserade cell som de framställts från och som ett resultat av detta kan de sedan lättare differentiera till samma celltyp som den de framställts från. Som exempel kan iPSC som framställts från blodceller mer effektivt differentieras till att åter bli blodceller, jämfört med iPSC som framställs från exempelvis huden. På samma sätt kommer iPSC från Hudceller ha lättare att differentiera till Hudceller än iPSC som genererats från blodceller. I min första artikel har vi utforskat denna teori och kommit fram till att effekten av så kallat celltypsminne på differentieringsförmåga är väldigt liten. Istället har vi visat att donatorns genom (nedärvda DNA) har en större påverkan på iPScellernas differentieringsförmåga. På grund av detta anser vi att iPS-cellbanker borde innehålla celllinjer från ett stort antal donatorer, och varje celllinje bör profileras för dess förmåga att bilda olika typer av specialiserade celler. Därför har vi, i min andra artikel, lagt fram förslaget att etablera en iPSC-bank med ett brett spektrum av celllinjer med olika HLA-typ och genombakgrund, detta via användning av celler från fostervatten som kan erhållas vid kejsarsnittsförlossningar. Tack vare det stora antalet kejsarsnitt som genomförs i många olika länder finns en enkel möjlighet att erhålla detta bredda spektrum av donatorceller med målet att generera olika iPSC-cellinjer. Genom att använda ett nytt system har vi också visat att uppsamling av signifikanta volymer av fostervatten är möjligt i samband med kejsarsnitt, vilket ger åtkomst till en stor mängd fostervatten-deriverade celler. Eftersom fostervattenceller är av neonatalt ursprung har de ännu inte utsatts för diverse mutagens ämnen i samma utsträckning som vuxna celler, och de har därför färre genetiska mutationer jämfört med celler från vuxna. Därför tror vi att denna neonatala källa till celler lämpar sig bättre som donatorceller för framställning av iPSC-cellinjer, eftersom det jämfört med att använda vuxna donatorceller minskar risken att en genetisk mutation går vidare via iPScellerna.

Skillnaden mellan män och kvinnor ligger i genomet. Det finns en uppsättning gener av vilka män endast bär en kopia, medan kvinnor ärver två kopior. Detta balanseras av att kvinnliga celler inaktiverar den ena kopian av dessa gener. Dock kan iPSC som framställts från kvinnliga donatorer under cellodling re-aktivera denna kopia av generna och därmed producera högre nivåer av dessa genprodukter än motsvarande celler från manliga donatorer. Det har föreslagits att denna så kallat icke korrekt nivå av genprodukter i kvinnliga iPSC kan orsaka dålig differentieringsförmåga. I min tredje artikel har vi utvärderat effekten av detta fenomen på kvinnliga iPSC-cellers förmåga att differentiera till blodceller och vi har
demonstrerat att re-aktivering av genkopian faktiskt har en negativ påverkan på blodcellsdifferentiering. Dessutom har vi kopplat denna nedsatta förmåga till blodcellsbildning hos kvinnliga iPS-celler till höga nivåer av en genprodukt vid namn ZIC3. Vi har föreslagit att den negativa effekt ZIC3 har på blodcellsdifferentiering medieras via en signalväg som visat sig vara viktig för bildning av blodceller. Sammanfattningsvis kan våra upptäckter hjälpa till att hitta lösningar för mer effektiv bildning av blod från iPSC, något som man hoppas kunna använda sig av för att behandla blodsjukdomar i framtiden.
جمع بندي علمي

سلول هاي بنيادي جنيني سلول هاي غير تخصصي هستند كه قادر به تبدیل به انواع سلول هاي بدن هستند. اين سلول ها درون بافت هاي جنيني يافت مي شوند و به عنوان منبع مستمر سلول هاي جديد در جنين عمل مي كنند. معادل اين سلول هاي بنيادي جنيني را مي توان در آزمایشگاه بوسيله ی دستکاری سلول هاي تخصصي توليد كرد. سلولهایی که توسط اين تکنولوژي توليد می شوند را سلولهای بنيادي پدنن پی دوران الگه (سلول های iPS) مي نامند. سلولهایی که توسط اين تکنولوژي توليد می شوند را سلولهای بنيادي پدنن پی دوران الگه (سلول های iPS) مي نامند. سلولهای iPS ميمكنند سلول های بيمار را توليد كرد و به عنوان يك مدل برای مطالعات بيماري هاي انسانی در يك ظرف از آزمایشگاه استفاده كرد. سلولهای این توانايي به تماسي سلول های بدن از جمله سلول هاي خوني را دارند و بنابارين مي توانند منبع بالقوه يك منبع مستمر سلول های جدید در جنين عمل كنند. معادل اين سلول هاي بنيادي جنيني را مي توان در آزمایشگاه بوسيله ی دستکاری سلول هاي تخصصي توليد كرد. سلولهایی که توسط اين تکنولوژي توليد می شوند را سلولهای بنيادي پدنن پی دوران الگه (سلول های iPS) مي نامند. سلولهای iPS ميمكنند سلول های بيمار را توليد كرد و به عنوان يك مدل برای مطالعات بيماري هاي انسانی در يك ظرف از آزمایشگاه استفاده كرد. سلولهای اين توانايي به تماسي سلول های بدن از جمله سلول هاي خوني را دارند و بنابارين مي توانند منبع بالقوه يك منبع مستمر سلول های جدید در جنين عمل كنند. معادل اين سلول هاي بنيادي جنيني را مي توان در آزمایشگاه بوسيله ی دستکاری سلول هاي تخصصي توليد كرد. سلولهایی که توسط اين تکنولوژي توليد می شوند را سلولهای بنيادي پدنن پی دوران الگه (سلول های iPS) مي نامند. سلولهای iPS ميمكنند سلول های بيمار را توليد كرد و به عنوان يك مدل برای مطالعات بيماري هاي انسانی در يك ظرف از آزمایشگاه استفاده كرد. سلولهای اين توانايي به تماسي سلول های بدن از جمله سلول هاي خوني را دارند و بنابارين مي توانند منبع بالقوه يك منبع مستمر سلول های جدید در جنين عمل كنند. معادل اين سلول هاي بنيادي جنيني را مي توان در آزمایشگاه بوسيله ی دستکاری سلول هاي تخصصي توليد كرد. سلولهایی که توسط اين تکنولوژي توليد می شوند را سلولهای بنيادي پدنن پی دوران الگه (سلول های iPS) مي نامند. سلولهای iPS ميمكنند سلول های بيمار را توليد كرد و به عنوان يك مدل برای مطالعات بيماري هاي انسانی در يك ظرف از آزمایشگاه استفاده كرد. سلولهای اين توانايي به تماسي سلول های بدن از جمله سلول هاي خوني را دارند و بنابارين مي توانند منبع بالقوه يك منبع مستمر سلول های جدید در جنين عمل كنند. معادل اين سلول هاي بنيادي جنيني را مي توان در آزمایشگاه بوسيله ی دستکاری سلول هاي تخصصي توليد كرد. سلولهایی که توسط اين تکنولوژي توليد می شوند را سلولهای بنيادي پدنن پی دوران الگه (سلول های iPS) مي نامند. سلولهای iPS ميمكنند سلول های بيمار را توليد كرد و به عنوان يك مدل برای مطالعات بيماري هاي انسانی در يك ظرف از آزمایشگاه استفاده كرد. سلولهای اين توانايي به تماسي سلول های بدن از جمله سلول هاي خوني را دارند و بنابارين مي توانند منبع بالقوه يك منبع مستمر سلول های جدید در جنين عمل كنند. معادل اين سلول هاي بنيادي جنيني را مي توان در آزمایشگاه بوسيله ی دستکاری سلول هاي تخصصي توليد كرد. سلولهایی که توسط اين تکنولوژي توليد می شوند را سلولهای بنيادي پدنن پی دوران الگه (سلول های iPS) مي نامند. سلولهای iPS ميمكنند سلول های بيمار را توليد كرد و به عنوان يك مدل برای مطالعات بيماري هاي انسانی در يك ظرف از آزمایشگاه استفاده كرد. سلولهای اين توانايي به تماسي سلول های بدن از جمله سلول هاي خوني را دارند و بنابارين مي توانند منبع بالقوه يك منبع مستمر سلول های جدید در جنين عمل كنند. معادل اين سلول هاي بنيادي جنيني را مي توان در آزمایشگاه بوسيله ی دستکاری سلول هاي تخصصي توليد كرد. سلولهایی که توسط اين تکنولوژي توليد می شوند را سلولهای بنيادي پدنن پی دوران الگه (سلول های iPS) مي نامند. سلولهای iPS ميمكنند سلول های بيمار را توليد كرد و به عنوان يك مدل برای مطالعات بيماري هاي انسانی در يك ظرف از آزمایشگاه استفاده كرد. سلولهای اين توانايي به تماسي سلول های بدن از جمله سلول هاي خوني را دارند و بنابارين مي توانند منبع بالقوه يك منبع مستمر S
سلولهای پوست تولید می‌شوند به طور موثرتری می‌توانند به سلولهای خون متمایز بگویند. به همین ترتیب، که از سلول‌های پوست تولید می‌شوند در مقایسه با خطوط می‌شوند به طور موثرتری می‌توانند به سلول‌های پوستی تبدیل شوند. در مقاله اول، ما این فرضیه را ارزیابی کرده‌ایم و نتیجه گرفته‌ایم که اثر حافظه نوع سلول بر توانایی تمایز خطوط iPS به سلول‌های مختلف جزئی است و با عوامل ارائه داده‌ایم که زن‌ها در کنار یکدیگر توانایی بیشتری بر توانایی تمایز خطوط iPS به سلول‌های مختلف را دارند. با توجه به تاثیر بیان زنان از این، کناره‌گیری در تمایز خطوط iPS با باید حاوی خطوط iPS از تعداد زیادی از این کناره‌گیری باشد و هر خاطه باید برای پاسخ‌گیری تمایز آن iPS نسبت به رده‌های سلولی متفاوت از ماشین‌شود. بنابراین، در مقاله دوم، پیشنهاد شده است که یک بانک با طیف گسترده‌ای از خطوط iPS با نوع زنی‌تکی با استفاده از سلول‌های مایع آمیویتکی که می‌توانند در هنگام زایمان سازارین به دست آیند، ایجاد شود. تعداد زیاد زایمان‌های سازارین در کشورهای مختلف، طیف گسترده‌ای از سلول‌های اکنون کنترل به پاس زمینه زنی‌تکی متفاوت‌تر را برای تولید‌سولول‌های ارائه می‌دهند. با استفاده از یک سیستم جمع آوری جدید، ما نشان داده‌ایم که بازیابی حجم قابل توجهی از سلول‌های آمیویتکی از زایمان‌های زن‌های سازارین می‌تواند با درک خزه‌های زمینه از تولید‌سولول‌های این سلول‌های منبع آمیویتکی می‌تواند به یک نشانده‌های مایع آمیویتکی مادیری که از منبع نوزادان مستند به اندامی سلول‌های بالغ در معرض عوامل جهش زا (ممتازان) قرار نگرفته وان برای بتا زنی‌تهیه که از منبع سلول‌های بالغ معیار سلول‌های نوزادان با نتیجه‌گیری متفاوت با استفاده از این سلول‌های بالغ بتوانیم این کناره‌گیری در iPS منبع سلولی بهتری برای تولید‌سولول‌های این کناره‌گیری در iPS طیف کارایی‌کرده‌ایم.

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