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REVIEW

Induced pluripotent stem cells: Mechanisms, achievements and perspectives in farm animals

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Abstract

Pluripotent stem cells are unspecialized cells with unlimited self-renewal, and they can be triggered to differentiate into desired specialized cell types. These features provide the basis for an unlimited cell source for innovative cell therapies. Pluripotent cells also allow to study developmental pathways, and to employ them or their differentiated cell derivatives in pharmaceutical testing and biotechnological applications. *Via* blastocyst complementation, pluripotent cells are a favoured tool for the generation of genetically modified mice. The recently established technology to generate an induced pluripotency status by ectopic co-expression of the transcription factors Oct4, Sox2, Klf4 and c-Myc allows to extending these applications to farm animal species, for which the derivation of genuine embryonic stem cells was not successful so far. Most induced pluripotent stem (iPS) cells are generated by retroviral or lentiviral transduction of reprogramming factors. Multiple viral integrations into the genome may cause insertional mutagenesis and may increase the risk of tumour formation. Non-integration methods have been reported to overcome the safety concerns associated with retro and lentiviral-derived iPS cells, such as transient expression of the reprogramming factors using episomal plasmids, and direct delivery of reprogramming mRNAs or proteins. In this review, we focus on the mechanisms of cellular reprogramming and current methods used to induce pluripotency. We also highlight problems associated with the generation of iPS cells. An increased understanding of the fundamental mechanisms underlying pluripotency and refining the methodology of iPS cell generation will have a profound impact on future development and application in regenerative medicine and reproductive biotechnology of farm animals.

Key words: Reprogramming; Large animal models; Stemness; Chimera; Germline transmission; Induced pluripotent stem cells; Gene delivery

Core tip: The generation of an induced status of pluripotency

in somatic cells by ectopic expression of core transcription factors allows to extending advanced genetic modifications and reproductive techniques to species, for which the derivation of genuine embryonic stem cells was not successful till now. The commonly employed viral gene transfer may be genotoxic and therefore non-viral methods for iPS cell derivation are intensively studied. In this review, we focus on the mechanisms of cellular reprogramming and current methods used to induce pluripotency.

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INTRODUCTION

Induced pluripotent stem (iPS) cells are defined as differentiated cells that have been experimentally reprogrammed to an embryonic stem (ES) cell-like state. The first generation of murine iPS cells was achieved[1] by retroviral transduction of four core reprogramming factors: Oct4, Sox2, Klf4, and c-Myc. Subsequently, human iPS cells were produced by viral transduction of adult fibroblasts[2,3]. Also a combination of Oct4, Sox2, Nanog and Lin28, was effective for the generation of human iPS cells[4]. An overview of reprogramming cells into iPS cells is shown in Figure 1.

Subsequently, the core reprogramming factors have been successfully used to derive pluripotent cells in various other species, including rhesus monkey[5], rat[6], pig[7], dog[8], cattle[9], horse[10], sheep[11], goat[12] and buffalo[13]. A summary of the generation of iPS cells from different species of livestock is enumerated in Table 1. Importantly, iPS cells could be isolated from several species, in which the isolation of authentic ES cells was not successful despite several attempts since many years[14,15]. In particular, for economically important species, such as farm animals, the availability of authentic iPS cells would have important consequences for reproductive biology and approaches for genetic modification. For agricultural purposes, iPS cells from farm animal species can serve as a valuable genetic engineering tool to boost the generation of livestock with advantageous genes that are important for economic, reproductive and disease resistant traits, or for the study of functional genomics in mammals.

So far, iPS cells have been successfully produced from fibroblasts[16], pancreas cells[17], leukocytes[18], hepatocytes[19], keratinocytes[20], neural stem cells[21], cord blood cells[22], and other cell types. Together these data suggest that most cell types can be reprogrammed to a pluripotent state, and that the unidirectional lineage commitment can be experimentally overwritten. Certain cell types, such as neuronal progenitors, which exhibit basal expression of one or more of the core reprogramming factors, seem to be ideal for reprogramming[21].

Rodent iPS cells are almost identical to their ES cell counterparts, sharing typical hallmarks of pluripotency such as colony morphology, unlimited self-renewal, *in vitro* and *in vivo* differentiation potentials, and contribution to the germline[23,24]. Most iPS lines from farm animal species have not been tested in chimera complementation assays; however some preliminary reports suggest that chimeras and germline transmission can be achieved in sheep and pig[25,26]. iPS cells derived from rodents, humans, monkeys and farm animals share the features of high telomerase activity, expression of alkaline phosphatase, and expression of stemness genes, such as *OCT4*, *SOX2*, *UTF1* and *REX1*. The epigenetic status of murine iPS cells has been analysed by bisulfite sequencing and chromatin immuno-precipitation DNA-Sequencing (ChIP-Seq)[27]. Thus the hallmarks for iPS cell characterisation can be enumerated as (1) unlimited self-renewal; (2) *in vitro* differentiation capacity; (3) *in vivo* differentiation capacity; (4) chimera contribution; and (5) subsequently germline transmission.

Apart from scientific and ethical hindrances, religious concerns restricted the derivation of human ES cells. To circumvent these concerns, alternative approaches to generate pluripotent cells have been assessed. The alternative approaches include culture of somatic cells with cell extracts isolated from ES cells[28] or oocytes[29], and fusion of somatic cell with pluripotent cell[30]. However, extremely low efficiencies, high technical difficulties and aberrant ploidies of the resulting cells[31,32] did reduce the enthusiasm for these attempts. At the moment, the derivation of iPS cells from human tissues seems to be the most promising alternative. Prior to clinical application of iPS-derivatives, cell survival, functional integration of the cellular transplant and safety of the cell products have to be assessed in informative animal models.

The progress in iPS cell development in farm animals lags behind those in rodents, but large mammalian models may be instrumental for pre-clinical tests of novel cell therapies (Table 2), enhanced pharmaceutical studies and regenerative studies, including the restoration of fertility.

HISTORICAL PERSPECTIVE

Ontogenesis of an organism and cellular differentiation were thought to be a unidirectional process, where stem and progenitor cells progressively develop to terminally differentiated cells, for example neurons, muscle, and epithelial cells. During ontogenesis the nuclear DNA of most cell types is unchanged, but different epigenetic marks, such as DNA methylation and histon modifications, are set, and lock the

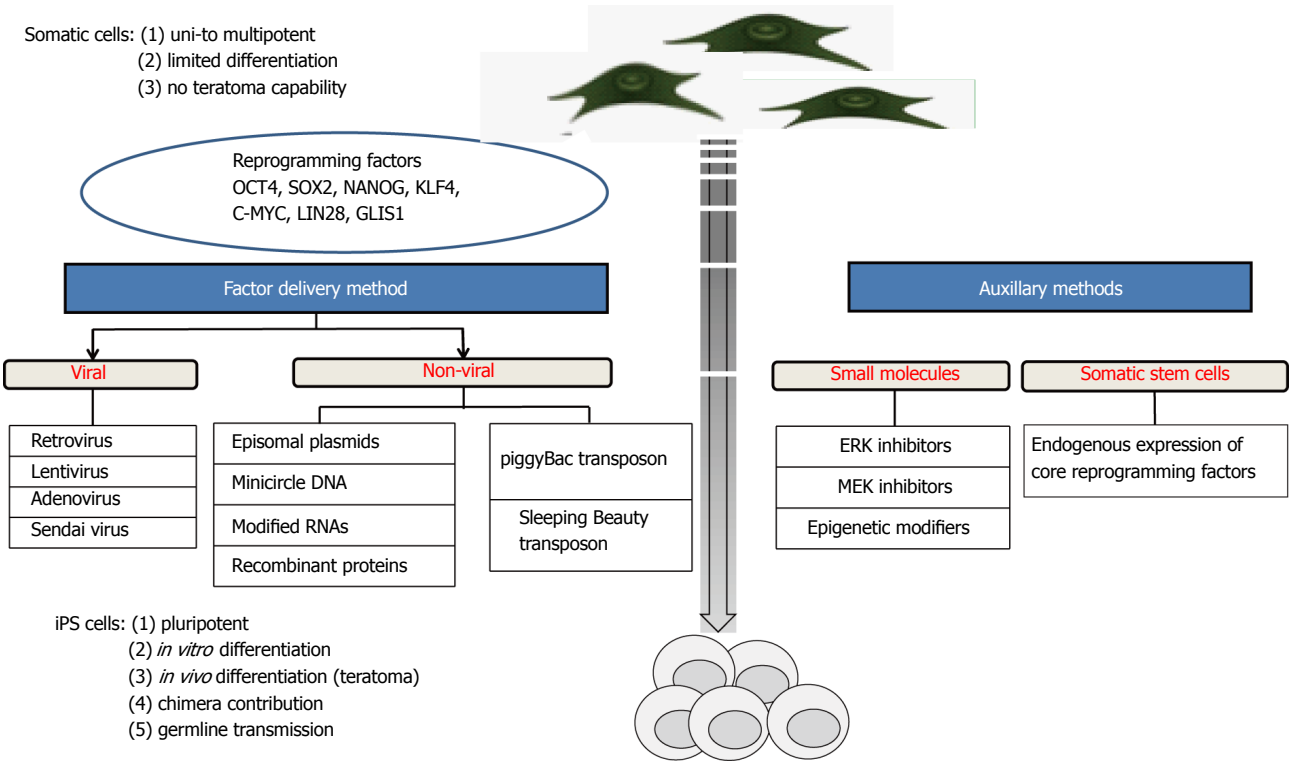

Figure 1 Methodological toolbox for generating induced pluripotent stem cells. iPS: Induced pluripotent stem.

cellular potency and cell lineage commitment. This is depicted by the "epigenetic landscape" proposed by Waddington[33].

Already in 1962, Gurdon[34] questioned this view by amphibian cloning; he transplanted nuclei from intestinal cells into irradiated oocytes and obtained vital tadpoles. More than three decades later, the successful cloning of a sheep (Dolly) by SCNT of a mammary epithelial cell to an enucleated oocyte, showed that even mammalian cells can be reprogrammed[35]. This success demonstrated that differentiated cells contain the genetic information to direct ontogenesis of an entire mammalian organisms, and that enucleated oocytes contain pivotal factors for reprogramming of differentiated cell nuclei. However, the identity of the oocyte reprogramming factors remained elusive.

The discoveries that ectopic expression of Antennapedia-a transcription factor was able and sufficient to induce leg structures in Drosophila[36], and that ectopic expression of the mammalian transcription factor MyoD1 converted fibroblasts into myocytes[37] led to the concept of "master genes". A master gene was defined as a key transcription factor that in a hierarchical manner regulates a cascade of critical genes, which in a concerted action induce the cell commitment.

DISCOVERY OF INDUCED PLURIPOTENCY

In 2006, Takahashi *et al*[1] proved that not a single master factor, but a a combination of four reprogramming factors, Oct4, Sox2, Klf4 and c-Myc, was sufficient to induce the pluripotent status in somatic mammalian cells. The resulting cells were called iPS cells[1]. This discovery offers new opportunities to study developmental biology, regenerative medicine, as well as reproductive biology and biotechnology of farm animals.

IPS cells from farm animals will likely serve as a bridging link between well developed rodent iPS and poorly characterised human iPS (Table 2), supporting the translation of innovative cell therapies from experimental studies to curative treatments. At the moment, human iPS cell application seems to be too risky because of basic lack of knowledge and ethical consideration which forbid certain tests such as chimera assays.

In contrast, research on iPS cells derived from farm animal species is not tainted with ethical concerns. Furthermore, the methodology for generation of iPS cells is relative simple and and is thought to be easily transferable to other mammalian species. Thus farm animal models may turn out to be ideally suited to determine required cell doses, to assess long-term performance, tumorigenicity, applications methods and fate of transplanted cells[38-41].

Recent advances in genetic engineering of farm animals allow the generation of precise genetic modifications[42-47], such as the production of immunodeficient pigs[48] which will be instrumental for further advances in preclinical testings of new cell therapies. A boost of recent

Table 1 Most advanced achievements in induced pluripotent stem cells from domestic animals

Domestic species	Cell type	Transduction	Reprogramming factors	Culture medium	Differentiation		Chimera	Germline contribution	Ref.
					In vitro	*In vivo*			
Buffalo	Fetal fibroblasts	Retrovirus	OSKM	A	EBs	Teratoma	NA	NA	[13]
Cattle	Fetal fibroblasts	Retrovirus	OSKM, OSKMLN, OSKM	B	EBs	Teratoma	NA	NA	[9]
	Fetal fibroblasts	Plasmid	OSKM	C	EBs	Teratoma	NA	NA	[53]
Dog	Skin fibroblasts	Lentivirus	Human OKSM	J	EBs	Teratoma	NA	NA	[56]
	Skin fibroblasts	Retrovirus	Mouse OKSM	K	EBs	Teratoma	NA	NA	[54]
Goat	Fibroblasts	Inducible lentivirus	OSKM, SV40 large T and hTERT	A	EBs	Teratoma	NA	NA	[12]
Horse	Fetal fibroblasts	PiggyBac transposon	OSKM	E	EBs	Teratoma	NA	NA	[10]
	Adult fibroblasts	Retrovirus	OSK	F	EBs	Teratoma	NA	NA	[61]
Pig	Mesenchymal stem cells from bone marrow	Lentivirus	OSNKLM	G	EBs	NA	Low grade	Two offspring	[26]
	Fetal fibroblasts	Sleeping Beauty transposon	Mouse OSKM	I	Neuronal lineage	Teratoma	NA	NA	[91]
Rabbit	Skin fibroblasts	Retrovirus	Human OKSM	I	EBs	Teratoma	NA	NA	[72]
Sheep	Fetal fibroblasts	Retrovirus	MKOS	D	EBs	Teratoma	Low grade	NA	[25]

A: DMEM, ESC FBS, L-glutamine, NEAA, β-Me, bFGF, LIF and MEFs; B: DMEM, KSR, L-glutamine, NEAA, β-Me, bFGF and MEFs; C: DMEM/F12 + N2 and Neurobasal with B27, L-glutamine, hLIF, PD0325901, CHIR99021 and MEFs; D: KO-DMEM, SR, L-glutamine, NEAA, 2-Me, human bFGF and MEFs; E: DMEM, FBS, L-Glutamine, NEAA, β-Me, Sodium Pyruvate, LIF, bFGF, Doxycycline, CHIR99021, PD0325901, A83-01, Thiazovivin, B431542 and 1:1 MEFs and EFFs; F: α-MEM, FBS, deoxyribonucleosides, ribonucleoside, glutamax, NEAA, β-Me, ITS, human LIF, βFGF, EGF and MEFs; G: DMEM/F12, KSR, L-glutamine, NEAA, β-Me, FGF and MEFs; H: KO DMEM, KSR, glutamax-L, NEAA, 2-Me, pLIF, forskolin and collagen I; I: DMEM/F12, KSR, L-glutamine, NEAA, β-Me, bFGF and MEFs or gelatinized plates; J: KO DMEM, ESC FBS, bFGF, hLIF and MEFs; K: DMEM/F12, KSR, bFGF, hLIF, PD0325901, CHIR99021 and MEFs. DMEM: Dulbecco's modified Eagle´s medium; LIF: Leukemia inhibitory factor; IGF1: Insulin-like growth factor 1; NEAA: Nonessential amino acids; FBS: Fetal bovine serum; KO: Knockout; MEM: Minimum essential medium; ITS: Insulin-transferring selenium; bFGF: Basic fibroblastic growth factor; DOX: Doxycycline; EB: Embryonic body; FCS: Fetal calf serum; hSCF: Human stem cell factor; KSR: Knockout serum replacement; MEFs: Mouse embryonic fibroblasts; OKSM: Oct-4, Klf4, Sox2, and c-Myc; OKSMLN: Oct-4, Klf4, Sox2, c-Myc, Lin28 and Nanog; VPA: Valproic acid; Me: Mercaptoethanol.

publications describe iPS cells from buffalo[13], cattle[9,49-53], dog[8,54-56], goat[11,57], horse[10,58-62], pig[7,63-71], rabbit[72-74] and sheep[11,75,76]. The majority of these iPS cells from farm animals showed typical hallmarks of pluripotency, such as differentiation *in vivo* and teratoma formation. However, most farm animal iPS cultures were not assessed for chimera contribution so far. Preliminary results that porcine iPS cells can contribute to chimera formation in blastocyst complementation were provided recently[71]. Similarly, ovine iPS cells contributed moderately to chimeric lambs after injection into eight-cell stage embryos or blastocysts[25]. These experiments represent an important step in the understanding of mechanistic nature of pluripotency in farm animals. The iPS technology may become instrumental for advanced transgenesis in large mammals (Figure 2).

METHODS TO DERIVE IPS CELLS

In recent years, several methods have been established for iPS cell generation (Figure 1), employing the core reprogramming factors as genes, mRNAs or proteins, and auxillary chemical agents, which infer with the involved signalling pathways. Here, the main approaches for the generation of iPS cells are summarized.

Virally-induced iPS cells

There has been extensive amount of work carried out to obtain virally-derived iPS cells employing either retroviruses, lentiviruses, and non-integrating viruses. The first iPS cells have been generated through retroviral transduction of Oct4, Sox2, Klf4 and c-Myc[1]. Disarmed, optimized retro- or lentiviruses can infect mammalian cells with high efficiencies. The use of the pantropic vesicular stomatitis virus G protein (VSVG) was instrumental for viral transduction of a broad spectrum of receptive cells. Interestingly, unstimulated T cells, B cells and hematopoietic stem cells could not be efficiently transduced with the VSVG lentiviruses[77].

Retro- and lentiviruses integrate into the host genome allowing for high expression of the encoded cargo genes. The expression can be temporally confined by employing viral promoters, such as the 5' long terminal repeat, which are usually silenced by epigenetic mechanisms. Disadvantages of the the viral approach include the limited cargo capacity of maximally 7 kb, the induction of immune responses and potential genotoxic effects. Retro- and lentiviral integrations do not happen randomly in the genome, but show a strong bias for promoter and exonic regions, which may result in dysregulation of endogenous genes. In a retrovirus-based clinical gene therapy of the X-linked

Table 2 Achievements with induced pluripotent stem cells from rodents, farm animals and humans

	Rodents	Farm animals	Human
iPS cells	√√	√√	√√
In vivo differentiation	√√	√√	√√
In vitro differentiation	√√	√√	√√
Chimera	√√	√/-	Ethically not allowed
Germline transmission	√√	√/-	Ethically not allowed
Follow up generations	√√	--	Ethically not allowed
Transplantation of iPS cell-derived cells	√√	√/-	No clinical studies to date[1]

√√: Fully proven; √/-: Partially proven; --: Not achieved yet; [1]The first clinical study was recently initiated (http://www.riken.jp/en/pr/press/2013/20130730_1). iPS: Induced pluripotent stem.

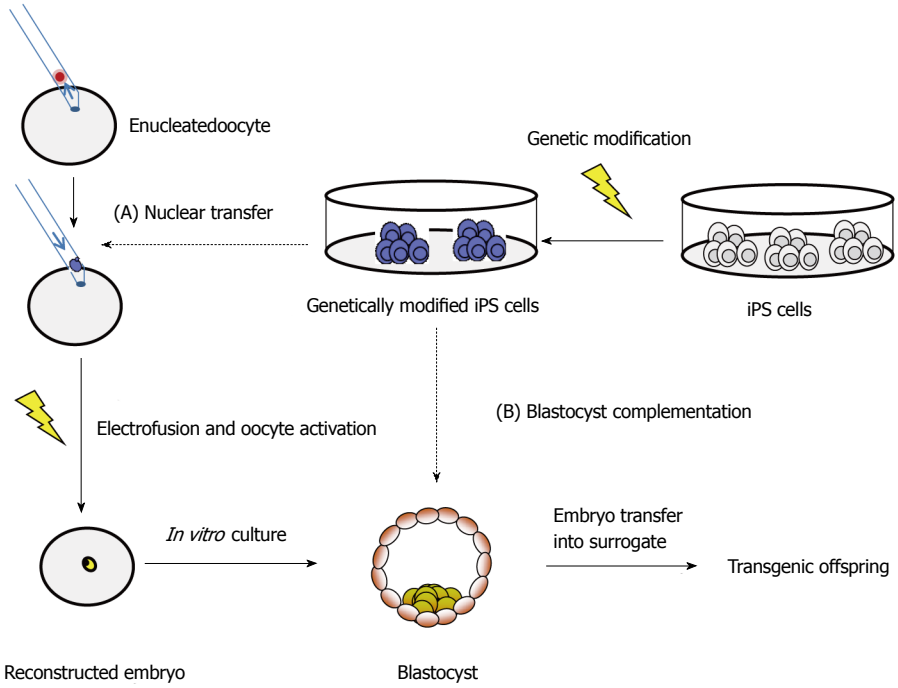

Figure 2 Application of induced pluripotent stem cells for advanced generation of transgenic animals. iPS: Induced pluripotent stem.

severe combined immunodeficiency (X-SCID), two of the treated children independently developed T-cell lymphomas due to viral integration in the neighborhood of the LIM domain only 2 gene[78]. These data highlight the risks of viral-based therapies[78]. Somatic cells derived from retrovirally reprogrammed iPS cells are apparently inconspicious, provided that the c-Myc transgene is silenced[19,79]. Retroviral reprogramming may evoke an immunogenicity of iPS cells[80]. Human iPS cell-like cells can be formed through transduction with lentiviruses, which do not carry reprogramming factors. The "pseudo" iPS cells were induced by viral encoded microRNA expression[81].

Alternative to integrating retroviruses, non-integrating adenoviruses can be used for reprogramming[17,82]. Another non-integrating virus is represented by the Sendai virus system. Sendai viruses enable efficient production of iPS cells and later on elimination of the viral vector[83]. Though viral mediated gene transfer offers high efficiency in generation of iPS cells, they require specific safety conditions for their handling.

Non-virally-derived iPS cells

The generation of iPS cells without viral transduction is preferable for regenerative medicine. Non-viral methods of reprogramming include episomal vectors[84], minicircle DNAs[85], plasmid vectors[86], small molecules[87], mRNAs[88], recombinant proteins[89] and transposons like piggyBac[90] and Sleeping Beauty[91]. In comparison to viral systems, non-viral approaches such as transposons are able to carry large DNA cargo into the host cell, they are non-infectious and do not evoke immune responses.

Episomal vectors

Episomal vectors for reprogramming of somatic cells were recently described[84]. In this method, reprogramming of fibroblasts was carried out by transfecting with the episomal vector oriP/Epstein-Barr nuclear antigen-1. This vector was chosen because it can be removed after reprogramming by a drug selection method. The iPS cells generated through this method show similar morphology and expression patterns to ES cells. Further, they were able to form

teratomas in immunocompromised mice. As there was no integration into the host genome, transgene free iPS cells may be selected through further sub-cloning. Despite these advantages, this method yields low reprogramming efficiency in human fibroblasts at about three to six iPS colonies per 10^6 input cells[84].

Minicircle vectors

Minicircle vectors are produced by the recombinatorial elimination of the bacterial backbone of the original plasmids. Minicircles containing the four reprogramming factors Oct4, Nanog, Lin28, and Sox2 in addition to an enhanced green fluorescent protein were used to obtain human iPS cells[85]. The group excised the bacterial backbone from the plasmid by taking advantage of the PhiC31-based intramolecular recombination system, which cleaves away the undesired bacterial plasmid backbones, leaving minicircle DNA to be purified containing the desired reprogramming factors[85]. It was claimed that minicircle DNA benefited from higher transfection efficiency compared to the parental plasmids. They also have longer ectopic expression, which is due to the lower activation of exogenous silencing mechanisms. Later, other groups reproduced the minicircle approach for reprogramming[92,93].

Small molecules

Nowadays, small molecules and chemicals are assessed to enhance reprogramming efficiency and iPS cell generation. The idea behind their use is to substitute core reprogramming factors with small molecules, which will serve to enhance the reprogramming. Shi *et al*[94] showed that neural progenitor cells, which endogenously express Sox2, were reprogrammed only by ectopic expression of Oct4 and Klf4. They also showed that this process was supported by the G9a histone methyltransferase inhibitor, BIX-01294 (BIX). Ichida *et al*[95] used small molecules (RepSox2) for replacing transcription factors (Sox2) by inhibiting transforming growth factor-β signalling. In this direction, Lee *et al*[96] used magnetic nanoparticle-based transfection method that employs biodegradable cationic polymer PEI-coated super paramagnetic nanoparticles for iPS cells generation. Recently, the L-channel calcium agonist, BayK8644, was assessed to improve generation of iPS cells[87] and it was claimed that BayK8644 does not directly cause epigenetic modifications as it works upstream in cell signalling pathways and can therefore avoid unwanted modifications. A more comprehensive list of small molecules involved in the iPS cells generation and their mechanism has been reviewed recently[97].

Transposon systems

The recent development of hyperactive transposase enzymes makes transposon systems an interesting alternative to viral based methods. The commonly employed Sleeping Beauty, piggyBac and Tol2 transposon systems are relatively simple organized, and the essential components can be separated on two plasmids. One plasmid carries the inverted terminal repeats (ITR) flanking the transgene, the other plasmid carries an expression cassette for the respective transposase enzyme. Upon co-transfer of both plasmids into a cell, the transposase becomes expressed and subsequently transposes the ITR-flanked transgene into the genome. Importantly, only the desired transgenes becomes integrated by a cut-and-paste mechanism, whereas the plasmid backbones are degraded. On a genomic scale transposon integrations appear to happen at random, without a bias for promoter and gene-containing regions. The integrated transposon can be removed seamlessly by supplying the transposase in trans[98], which makes the system more attractive and relevant in producing the safe and clean iPS cells. Up to six reprogramming factors have been connected by self-cleaving peptide sequences allowing for coexpression from a single cassette[91,99-103]. Individual proteins are then produced by the self-cleaving peptide[104-106].

Reprogramming with protein factors

The discussed transposon and episomal systems still require the introduction of cargo DNA into the cells[106]. Delivery of reprogramming factors as proteins is an obvious alternative. In 2009, transgene-free iPS cells were produced with proteins of reprogramming factors[107]. Therefor recombinant reprogramming proteins were produced as fusion proteins containing cell penetrating peptides. Repeated supplementation of the culture media of fibroblasts converted them to iPS cells. However, the protein-based reprogramming approach has not found widespread use, mainly due to relative low reprogramming efficiencies, and high costs for repeated treatments with protein factors.

mRNAs and microRNAs

The most recent trend in the field of non-viral iPS generation is reprogramming by using RNA molecules. Recently, modified mRNAs encoding reprogramming factors were employed to generate iPS cells with high efficiency[108]. Messenger RNAs are an ideal vehicle for reprogramming, because they do not bear the risk of integrational mutagenesis, they can be transduced to cells with high efficiency, and they can be combined in desired ratios of the individual factor encoding transcripts[108]. Disadvantages of mRNAs are the short half-life of -10 h, and that innate immune responses must be inhibited to allow for the full effects[109].

Recently, it was shown that micro RNAs (miR) expression is sufficient to induce pluripotency[110-112]. Two independent groups reported iPS cell generation by delivery of miR302, or miR200c, miR302, and miR369[113,114]. These miR-derived iPS cells were indistinguishable from conventionally generated iPS cells. MicroR reprogramming seems to have advantages

for cellular reprogramming[114-116], for example it avoids the need of transducing proto-oncogenic transcription factors[117,118]. However, it needs to be assessed whether this approach will be successful in other species, since the underlying mechanisms are not well understood[119].

MOLECULAR FACTORS REGULATING REPROGRAMMING

The core factors for reprogramming are Oct4, Nanog, Sox2, Klf4, c-Myc and Lin28. These genetic factors reprogram cells by regulating critical signalling pathways, epigenetic modifications and micro RNAs[114].

Reprogramming by core transcription factors

Oct4 is the best studied regulator of pluripotency. Oct4 expression is confined to early embryonic cells, germ line cells and cultured pluripotent stem cells, where it activates the gene transcription of stemness gene[120]. Oct4 protein cooperates with stemness factors such as Nanog and Sox2, but it also interacts with Polycomb group proteins[120], which are important repressors of transcription. Sox2 is a transcription factor that acts as coactivator of Oct4[121]. Binding of Oct4/Sox2 dimers to the promoter sequences of *Oct4* and *Nanog* genes upregulate their transcription[122]. Nanog is a homeobox-containing transcription factor stabilizing the stemness network[122]. Klf4 is a zinc finger-containing transcription factor which regulates the expression of Oct4, Sox2 and Nanog[123-125]. Over-expression of Klf4 in ES cells increased the expression of Oct4 which further improve the self-renewal ability[126]. c-Myc enhances the efficiency and speed of reprogramming[127]. LIN28 promotes the expression of Oct4 at the posttranscriptional level by direct binding to its mRNA[128]. Recently, Glis1 has been identified as a substitute for c-Myc[129]. Glis1 transactivate the genes of Wnt ligands, Lin28a, Nanog, Mycn, Mycl1, and Foxa2[129].

The aspect of whether the species-specificity of reprogramming factors is relevant for proper reprogramming, is not well understood. In principle, the essential domains of the reprogramming factors are highly conserved between mammalian species, and several publications showed successful reprogramming with human and murine sequences in other species[5-13,130].

APPLICATIONS OF IPS CELLS

Modeling of human diseases and preclinical trials

The potential applications of iPS cells will impact regenerative medicine, pharmaceutical industry, and animal biotechnology[131]. Human iPS cells could be utilized for curative treatments, to studying onset and disease progression *in vitro*, and to test potential therapeutic in high throughput screens[114,131,132]. The production of disease-specific iPS cells has found widespread use in recent years[133-136]. Disease-specific iPS cells provide a unique resource to obtain a molecular understanding of disease onset and progression[131,132]. Induced PS-derived differentiated cells will allow to carry out *in vitro* drug screening (Figure 3), and to test therapeutic interventions[131]. In mice, Fanconi anemia and sickle cell anemia have been successfully corrected by using iPS cells[131,133-136].

However with regard to potential curative treatments, the functionality, safety, and lack of tumorigenicity of iPS-derived cells have to be assessed in appropriate animal models bearing significant physiological and anatomical similarities to humans (Table 2). Hence, animal models could be contributed tremendously to a better understanding of disease mechanisms and therapeutic interventions. In addition, iPS cells from monkey[5], porcine[41,26], canine[8] and cattle[9] would be useful in animal biotechnology such as making precise genetic engineering for improved production traits and products[137,138].

Advanced transgenesis in large mammals

Transgenic farm animals can serve as excellent models of human diseases and during the past few years transgenic farm animals have gained renewed popularity. This is due to the availability of annotated genome depositories of the major domestic species and other organisms (for example: www.ensembl.org; or www.ncbi.nlm.nih.gov/genome), and due the introduction of active methods of transgenesis, which dramatically increased the success rates[42,43]. The repertoire of molecular tools now allows the precise modification of large mammalian genomes at rapid pace and has led to a recent boost in this area. The development of genuine iPS cells from domestic species will contribute to these advances and allow to perform desired genetic modifications *via* high throughput screens *in vitro*, and then use either SCNT[47] or blastocyst complementation for the generation of transgenic offspring (Figure 3). However at the moment most of the iPS cells cultures from different domestic species have not been tested for their capability to contribute to chimera formation, and only preliminary data are available[25,26]. Thus reinforced efforts to assess the potential of current livestock iPS cell lines for chimera contribution and germ cell differentiation are required. The majority of current livestock iPS cell lines are generated with retro- or lentiviral reprogramming approaches (Table 1), and the opportunities to assess alternative non-viral approaches are not widely assessed[10,56,106]. Also the potential of auxillary small molecular inhibitors of stemness signaling pathway is not exploited for livestock iPS cells. Potentially, high throughput screens to identify small molecules with species-specific activity are required. It is anticipated that these approaches will lead to livestock iPS cells, which will make a significant impact for future genetic modifications of these species.

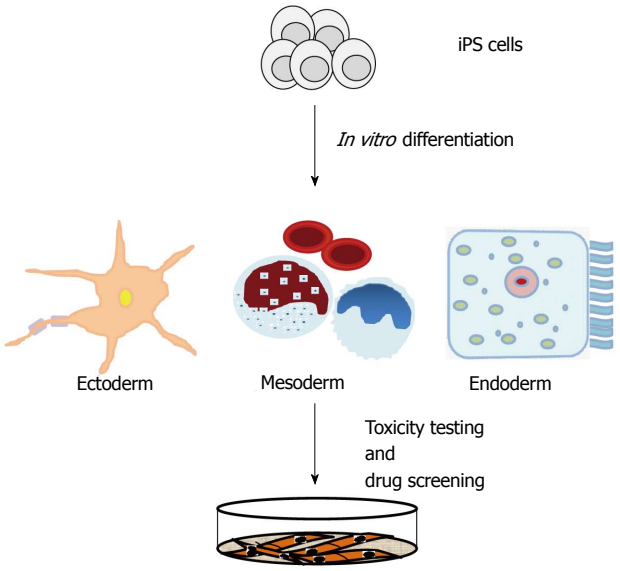

Figure 3 IPS cell technology contributes to disease modelling and drug discovery. iPS: Induced pluripotent stem.

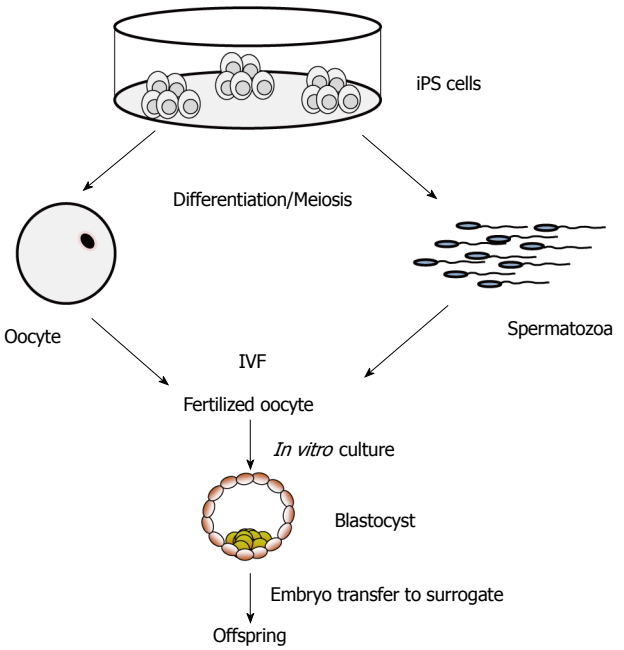

Figure 4 Application of induced pluripotent stem cells for *in vitro* generation of gametes. iPS: Induced pluripotent stem; IVF: *In vitro* fertilization.

Preservation of genetic resources and endangered breeds

The iPS technology has the potential to preserve endangered animals and highly valuable genotypes in the near future[139]. Cryopreservation of cells and tissues is an important and useful approach for genetic preservation of valuable breeds and for conservation of endangered wild and domestic species. For highly endangered species, the derivation of iPS cells may become a method to prevent extinction. For example, iPS cells have been produced from endangered snow leopard[140], drill and white rhinoceros[139]. The iPS cells generated can be easily expanded for banking of genetic material, or used as donor cells for SCNT. Potentially, iPS cells from endangered species may be differentiated into mature oocytes and spermatozoa (Figure 4), which might be employed for *in vitro* embryo production[139,140]. The differentiation of livestock iPS cells to functional gametes *in vitro* have not been achieved yet, however the current pace in developing fine-tuned protocols for *in vitro* differentiation of desired cell types, and the progress in inducing meiosis support the notion that the generation of fully functional spermatozoa and oocytes may be feasible. The possibility to obtain fully functional spermatozoa and oocytes from iPS cells of domestic and wild species would has far reaching consequences for maintenance of endangered species, as well as for breeding and genomic selection programs of domestic species. Even potential applications for infertility treatments in humans may become feasible[141,142].

PROSPECTS OF FARM ANIMAL IPS CELLS IN PRECLINICAL STUDIES

The generation of iPS cells has opened new vista to understand pluripotency, disease onset and progression, and to develop regenerative medicine[132]. However, before the clinical application of iPS cell-derived therapies can be envisioned, the low efficiency and kinetics of iPS cell formation, the risks of insertional mutagenesis, reactivation of silenced ectopic transgenes and potential tumor formation have to be assessed and solved[131]. An important aspect is the biosafety of transplanted derivatives of iPS cells[132]. A number of reports showed that iPS cell lines could contain genetic mutations, copy number variations, and epigenetic mutations[132,143-145]. These aberrant changes may increase the tumorigenicity of iPS and iPS-derived cells. Retro- and lentiviruses are commonly used to introduce the reprogramming factors into differentiated cells, which can increase the immunogenicity[146].

Farm animals represent informative model organisms, which seem to be suitable to assess obstacles and risks in longitudinal pre-clinical studies[147]. In contrast to rodent models, they are more similar to humans with respect to life-span, physiology, metabolism and pathophysiology[148,149]. Large mammalian models will allow to determining required cell doses to obtain therapeutic effects, to follow the fate of transplanted cells and their functional integration in the host tissue[150]. Thus the research on pluripotent stem cells from farm animals will contribute to the development of innovative cells therapies for human patients.

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