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Review Article

A Brief Introduction for Source and Application of Chicken Pluripotent Cells-A Narrative Review

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Abstract

Pluripotent cells such as embryonic stem cells or Mesenchymal stem cells are kinds of undifferentiated cells and have the capable of self-renewing and can differentiate all kinds of cells including somatic cells and the germ line. Pluripotent cells are an excellent cell model for embryonic development research and regeneration medicine. So far, there are two kinds of chicken pluripotent cells (chicken embryonic stem cells and chick primordial germ cells). Chicken embryonic stem cells comes from stage X blastoderms and have the ability to differentiate into embryoid or the three primitive germ layer just like mammalian embryonic stem cells and chicken primordial germ cells are a kind of unipotent stem cells only differentiated into the germ line. There are many exciting research progress on chicken pluripotent cells in the recent years. In this review, we introduced basic characteristics, sources, isolation methods and culture method of chicken pluripotent cells, application of chicken pluripotent cells on manufacture of viral vaccines and production of chimeric chickens were also discussed.

Keywords: *Chick Embryonic Stem Cells; Chick Primordial Germ Cells; Viral Vaccines; Chimeric Chicken*

Introduction

Bird embryos have been a powerful model for development and stem cell research over the past few decades because of convenient source and suitable size [1]. Nowadays, chicken is the only non-mammalian animal that established stable embryonic stem cell and germ cell. There are two main chicken pluripotent cells, chicken embryonic stem cells (cESCs) and chick primordial germ cells (cPGCs) [2-4]. cESCs have been proved to be able to differentiate into all kinds of somatic tissues except the germ line and only cPGCs can contribute to germ line [5].

The first 20 hours of chick embryos is in the womb then the shell is deposited as the egg descends along the maternal fallopian tube [6]. The embryos become a disc with a single-cell-thick layer during this time and contained about 20,000 - 50,000 cells [7]. The entire embryo will emerge from the center of the outer embryo, but it retains its remarkable ability to regenerate. The blastoderm fragment can regenerate the whole embryo and re-establish polarity [8,9]. In this stage, stem cells can dissociate from the central epiblast and those cells can culture indefinitely, which like mammalian embryonic stem cells [10,11].

The biology of chick embryo germ cells is specifically absorbing and unique. Primordial germ cells (PGCs) appear to be produced at a pre-primordial streak stage (see above) and combining with the lower germ cells below [12]. PGCs migrate to germinal crescent through embryonic blood and eventually contributed to embryonic gonads [13,14].

The major application of chicken embryonic stem cell technology is generated somatic and germline chimeras in the past decades [15]. The unique self-renewal characteristics and lack of transforming oncogenes and exogenous factors make cESCs an ideal cell matrix for the production of viral vaccines [16,17]. The vaccine industry has a length and expanded history of using chick substances, so as ethical and safety issues related to mammalian embryonic stem cells have emerged, the focus has shifted to the use of avian embryonic stem cells for vaccine production [18,19].

Nowadays, it has been probable to create long-term, self-renewal cell cultures from blastoderm stem cell and germ cells isolated from the vascular system or gonads. Some cell lines have also been successfully established from later embryonic and adult tissues [20,21]. This review summarized our current understanding of stem cells from these different sources and their key biological properties.

Sources of chicken pluripotent cells

Chick embryonic stem cells

A lots of the current stem cells research is done using mammalian cells (such as mice and human) *in vitro* [22]; chicken is the only non-mammalian systems that have been able to establish pluripotent embryonic stem cell lines in avian system [23]. Cells isolated from the blastoderm of a stage X fertilized egg are often referred to as cESCs [24], which have a relatively small quality of cytoplasm and a large translucent nucleus [25]. cESCs, same as mammalian embryonic stem cells, have the characteristics of self-renewal and multidirectional differentiation potency [26]. They can express a number of pluripotent markers including stage specific embryonic antigen-1 (SSEA-1) and alkaline phosphatase (AKP) and can produce teratomas with three germ layers *in vivo* and embryo-like body *in vitro* [27] (Figure 1).

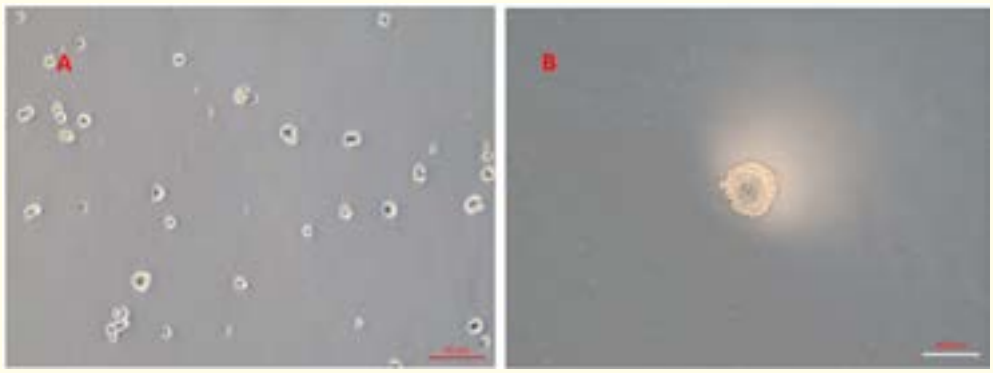

Figure 1: *The cell morphology and embryoid body of cESCs.*

cESCs can be isolated from the blastoderms, usually taken out blastoderms and washed with phosphate-buffered saline and mechanically dispersed [28,29]. There are four methods to obtain blastoderm including spoonful method, paper ring method, hair circle method and whole embryo extraction method [30]. Of all the four method, the blastoderm isolated from the hair ring method carried less yolk, on the contrary, the spoon method or the whole embryo extraction method are easily contaminated for contain a large amount of egg yolk [31].

Those cells were placed on inactivated feeder cells in the presence of suitable media [32]. The medium was changed daily and after 3-4 days, the cESCs-like colonies were dissociated. The colonies were spread on a new 24-well culture dish covered with inactivated MEF cells [33].

To date, it has been evaluated for long-term maintenance of cESCs by using of various media, different feeder layers, various growth factors, different kinds of serums and different chicken strains [34-36]. Like mESCs, growth factors and cytokines, such as CNTF, OSM and LIF, bFGF, IGF-1, mSCF, IL-6, IL-11, were added in embryonic stem cell medium [27]. As a member of the interleukin-6 (IL-6) family, Leukemia inhibitory factor (LIF) was initially identified as an inducer of M1 leukemic cell and was later found to be an inhibitor that can control the differentiation of embryonic stem cell [37,38]. During early embryonic development, one of the most important growth factors is FGF4, which mainly maintains pluripotency of embryonic stem cells through PI3-K/Akt signaling pathway and sarcoma/MEK/extracellular signal-regulated kinase signaling pathway [39,40].

Chick primordial germ cells

Of all the cell types, only germ cells have the ability that passes on genetic information to the next generation by gametogenesis. Germ cells do not appear in the gonads in the beginning [41-43]. The germ cell lineage is separated from all somatic cells in the embryo during early development and this kinds of cells is described as primitive germ cells (PGCs). Waldeyer is the first one found chick PGCs in 1870. The main biological characteristics of avian PGCs are as follows: in the first place, their unique migration pathway by the blood circulation to the genital ridge, in the second place, their ability of long-term culture *in vitro*, which is only differentiated to the germ line [44,45].

Swift thought chicken germ cells were primarily originating in the hypoblast in 1914 [46]. But in 1981, Eyal-Giladi showed avain germ cells derived from epiblast through experiment. PGCs have the morphological characteristics with an eccentrically positioned, big nucleus and multiple lipid granules in cytoplasm [47]. So, when they migrate to genital ridge through blood vessel, it is not difficulty distinguished from erythrocytes. Chicken PGCs can be detected by periodic acid-Schiff (PAS) reaction for it contain amount of glycogen in the cytoplasm [48,49]. To date, germ-specific molecular markers for avian germ cells have been studied. The vasa gene is a DEAD-box RNA helicase and has been regarded as a specific marker of germ cells of chicked. RNA-binding protein, DND1 and DAZL are chicken germ cell molecular marker [50,51].

Germline chimeric chicken were generated in 1993 by transplanting cPGCs into a recipient chick embryo. But in this time, cPGCs cannot obtain too much for there was no technique to mass propagate PGCs *in vitro* at the time [51,52]. Chang., *et al*. (1995) used a medium with insulin-like growth factor 1 (IGF-1), fibroblast growth factor 2, leukemia inhibitory factor (LIF) and serum cultured chicken PGCs *in vitro* over 4 days [53,54]. This method can only be applied to cell conservation for the growth of PGCs is so slow that cannot long-term culture. Until in 2006, the long-term culture of chicken PGCs was succeeded by cultured on the feeder of STO fibroblast with medium conditioned LIF, FGF2, SCF, IGF and serum [55]. The application of N2B27/R2i+LIF medium validated the efficacy of identified pluripotency supporting medium for the effective derivation of chicken PSCs [33] (Table 1).

Component	Function/component
DMEM/F12	Medium
Neurobasal Medium	Medium
N2 Supplement	Medium
B27 Supplement	Medium
25% Bovine Serum Albumin	Serum
PD0325901	MEK inhibitor
CHIR99021	GSK3 inhibitor
Gentamycin	Antibiotics
LIF	Leukemia Inhibitory Factor

Table 1: *N2B27/R2i +LIF medium.*

Applications

Manufacture of viral vaccines

Embryonated eggs and their derived primary chicken embryonic fibroblasts (cEF) remain the main avian substrates for the study of avian viruses and for the production of vaccines, both in avian and human [56]. However, there are many limitations of cEF cells, such as high cost, safety of supply, reproducibility between batches, risk of occasional infection and lengthy production processes. Pluripotential cell lines are genetically relatively stable and have an infinite life cycle, potentially providing faster, more cost-effective and higher yield cell culture systems [57]. cESCs have been derived from early chick blastoderms (stage X). The grow and maintain of cESCs relatively easily in culture [58] and their phenotypic characteristics can be maintained after extensive amplification [59]. The unique self-renewal characteristics and lack of transforming oncogenes and exogenous factors make embryonic stem cells an ideal cell matrix for the production of viral vaccines [60]. The focus has shifted to using embryonic stem cells from birds to produce vaccines given the ethical and safety issues associated with mammalian embryonic stem cells. For example, the Japanese health authorities has authorized the use of the duck embryonic stem cell line EB66 to produce H5N1 influenza vaccine. In addition, cESCs EBX cell lines EB14 and EB45 have shown promising cell growth and productivity against a variety of viruses in bioreactors [61,62].

Currently, the standard avian cell matrix used in the study of avian influenza viruses is the cEF-derived spontaneously immortalised DF-1 fibroblast cell line [63,64]. The limited of those spontaneously immortalised cell lines has been a critical problem for diagnosis and research in the field of avain virology. cESCs can be a *vitro* model system for studying host response to vaccine viruses. cESCs can product more vaccinia virus Ankara (MVA) than DF-1 cells and are useful to gain fowlpox virus (FP9), canarypox virus (CNPV) and IFN-a [65].

Production of chimeric chickens

To improve the characters, such as meat, egg yield and disease resistance, pluripotent stem cells (primordial germ cells and embryonic stem cells) have been established and used [66]. Pain is the first one reported the methods for isolation and derivation of cESCs lines. They established cell lines using cells obtained from the blastoderm of chicken embryos before the formation of gastrulation (stage IX-XI) and dissociated in ESA medium mechanically [67,68]. Chicken PGCs can be isolated and cultured from pre-primitive-streak stage embryos. It migrates to the embryonic gonad by blood circulation, which is different with mammalian [69,70].

Retroviral vectors have been used to transfer exogenous genes into chick embryo blastoderms and somatic cells to produce chick chimeras, but transgenic transmission by germline has not been shown [71,72]. On the contrary, lentiviral vectors have been used quite successfully to produce transgenic strains of several birds, including chickens and quail. This method is almost routine now, a few lab is being establishing bird's transgenic strains, such as the train universal expression of green fluorescent protein. Despite the success of the construction of transgenic birds, lentiviral transduction is not easy to target mutagenesis at selected gene sites because the transgenes are inserted randomly at multiple sites [73,74].

Cells derived from the blastoderm were first used to make chimeric chickens after transplantation into the central region or the subblastoderm of early embryo [74,75]. There has been some success of the generation of somatic and germ line chimeras with early blastoderm cells containing PGCs from stage X embryos. While the limited plasticity of these chicken cells both cPGCs and cESCs are difficult to maintain in long-term culture and their ability to produce transgenic animals are highly variable [1,76].

Induced pluripotent stem cells (iPSCs) are a new class of pluripotent cells that have proven robust, can successfully develop transgenic mice, rats, quails and pigs and have the potential to overcome the limitations of previous chicken pluripotent stem cell systems [77]. In a study, researchers used a non-viral minicircle reprogramming method to generate chicken iPSCs from fibroblasts cells. ciPSCs expressed

key stem cell markers, such as POU5F1, SOX2, alkaline phosphatase, Nanog and SSEA-1 and demonstrated stem cell morphology. ciPSCs transplanted into the stage X embryo were successfully integrated into the tissues of all three germ layers and the gonads showed significant cellular plasticity [78]. The method and cells provide an exciting new tool for creating transgenic chicken [79].

Conclusion

Stem cell biology is a speedily growing field that now includes not only embryonic stem cells and embryonic germ cells, but also a small number of adult stem cells. There have been many efforts to develop pluripotent cells in mammals other than mice; However, none of them can produce germline chimeras. Chicken pluripotent stem cells give rise to germline chimeras. Although chick embryonic stem cells and embryonic stem cell germline chimeras less efficient, it is appearing to be optimal for early generation and the potential for using avian systems will expand as our understanding of pluripotency. Pluripotential cell lines, which are genetically relatively stable and have an infinite lifespan, have the potential to provide faster, safer, more cost-effective and higher yield cell culture systems.

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Author Contributions

Conception and design: Haibin Ma, Junzheng Yang.

Manuscript writing: Haibin Ma, Junzheng Yang.

Final approval of manuscript: Haibin Ma, Junzheng Yang.

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