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A Glimpse of Stem Cell Research in China

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2010 has seen rapid progress in stem cell research in China. Not only the major funding agencies had provided extensive funding to stem cell research, the publications by Chinese stem cell biologists also flourished in top-notch scientific journals. In this special review, we highlighted some recent studies that had been published in *Science China Life Sciences* and *Cell Research*, two key SCI journals based in China. Not surprisingly, these studies mainly focused on some of the highly pursued stem cell types: embryonic stem cells (ES cells), induced pluripotent stem cells (iPSCs), neural stem cells (NSCs) and mesenchymal stem cells (MSCs).

1 Research on embryonic stem cells

ES cells are derived from the inner cell mass of blastocysts and are capable of infinite self-renewal and differentiation into various cell lineages within the three germ layers. A fundamental question in stem cell biology is what are the molecular mechanisms underlying lineage specifications along the developmental process. To study the transition of cell fate from A to B, it is important to use a system that can monitor the cell identity in an accurate and timely manner. Ho *et al.* found that, compared with protein markers and gene expression profiling, a change in the cell signaling network more sensitively reflects cell fate transition during the early differentiation of mouse ES cells [1]. In this study, the authors compared the mouse ES cells with the 2-day embryoid bodies (EBs) in terms of expression of stem cell markers (Oct4, Sox2 and Nanog etc.) as well as the cell signaling networks in response to extracellular stimuli, leukemia inhibitory factor (LIF) and fibroblast growth factor 8 (FGF8). mRNA levels of Oct4, Sox2 and Nanog and protein levels of Oct4 were not significantly different between mouse ES cells and up to 4-day EBs. Using phospho-specific antibody arrays and Western blot, the authors examined the activity of 8 signaling nodes (Erk1/2, p38, Akt1/2, Gsk3α/β, Stat3, β-catenin, Smad1, and Smad2) that play critical roles in mouse ES cell functionality, and found a clear difference in the signaling network status between ES cells and 2-day EBs when probed with LIF or FGF8 stimulation. The authors proposed that the cell signaling network may represent a fundamental characteristic of a cell and can more precisely reveal the cell identity and predict the cell behavior under certain perturbations. However, a drawback of this system is that it is based on the examination of a population of heterogeneous cells and cannot monitor cell fate at a single cell level, which could be very instrumental in studying the very early embryo development. In addition, live cell staining with specific markers may also be preferred when real time dynamics of a cell needs to be studied.

One important component of the signaling network in a stem cell is TGF-β family signaling, which plays crucial roles in the regulation of ES cell self-renewal and differentiation [2]. In an article published by Fei and Chen, the authors summarized the roles of TGF-β family (TGF-β, bone morphogenetic protein (BMP), Activin and Nodal) in different aspects of an ES cell behavior that includes self-renewal and pluripotency maintenance, as well as ES cell differentiation into the ectodermal, mesodermal, endodermal and trophectodermal lineages. Particularly, Chen *et al.* found that Smad2-mediated Activin/Nodal signaling is

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dispensable for the self-renewal of mouse ES cells, but is critical for the mesendodermal differentiation, with Tapbp as a key downstream player[3].

In another study published by Bai *et al*.[4], the authors attempted different approaches to promote cardiomyocyte differentiation from human ES cells, and found that treatment with 0.1 mmol/L ascorbic acid alone, or more notably in combination with 10 μmol/L 5-aza-2-deoxycytidine, can dramatically increase the number of beating clusters within EBs —— a readout for cardiomyocyte specification.

A study reported by Li's lab[5] demonstrated an interesting correlation between the metabolic properties and the states of chicken embryonic stem cells. Li *et al*. found that undifferentiated chicken ES cells maintain a high glycogen level by directing glucose flux towards the glycogenic pathway. However, upon the commencement of differentiation programs, the metabolism is switched from a glycogenic to a glycolytic pathway, suggesting that the cellular level of glycogen may indicate a chicken ES cell state. In addition, supply with glucose-6-phosphate can enhance the survival of chicken ES cells *in vitro*.

2 Research on induced pluripotent stem cells

iPSCs are pluripotent cells derived from a non-pluripotent cell, normally a somatic cell, by forced expression of defined factors. iPSCs are similar to natural ES cells in many aspects and meanwhile retain almost identical genetic information of donor cells and can also avoid the controversial use of embryos[6-7]. Through tetraploid complementation assay, Zhou *et al*. showed that iPSCs can produce viable mice, which confirms the true pluripotency of iPSCs and opens a new avenue for animal cloning[8]. However, only a very small fraction of mouse iPSCs can pass the tetraploid complementation test and give rise to live offspring. To increase the efficiency of animal cloning, Zhou *et al*. combined the two approaches, iPSC and nuclear transfer (NT) techniques[9]. While no mouse embryonic fibroblast (MEF)-NT (nuclei of MEF transferred to enucleated metaphase Ⅱ oocytes) embryos developed into live animals, about 1% of iPSC-NT embryos gave rise to live mice. This combined approach may provide a new route to obtaining cloned animals from resistant donor species and facilitate the generation of transgenic animal models.

A drawback of the iPSC technique when it was first reported by Yamanaka *et al*. was the low efficiency[10]. To induce mouse iPS cells, MEFs can be used as the start cells and be infected with virus encoding the "Yamanaka factors", Oct4, Sox2, Klf4 and c-Myc. After that, the infected MEFs are cultured on feeder cells in the induction medium until the iPSC colonies emerge. Mouse ES cell growth medium containing 15% fetal bovine serum (FBS) is commonly used as the induction medium to facilitate the reprogramming process of MEFs. Zhou *et al*. found that replacement of FBS with 20% of knockout serum replacement (KOSR), a commercially available supplement, can significantly accelerate the reprogramming process, as well as augment the efficiency by more than 200 fold (~24% with KOSR versus ~0.1% with FBS at Day 20 post-infection)[11]. In addition, the iPS cells obtained by this method are of good quality and possess full developmental potentials including germ-line contribution in chimeric mice and full-term development of tetraploid complementation embryos.

3 Research on neural stem cells

In vertebrates, gastrulation creates an embryo with three germ layers. The interaction between the dorsal mesoderm and the overlying ectoderm directs the ectoderm to form the hollow neural tube, which will differentiate into the brain and spinal cord. Epigenetic regulation has been shown to play important roles in the development of the nervous system. In a recent report, Chen *et al*. discovered a new histone demethylase, KIAA1718 (KDM7A), which is specific for histone H3 lysine 9 and lysine 27 —— two epigenetic marks associated with transcription repression. Expression of KIAA1718 increases along with the neural differentiation of mouse ES cells and knockdown of KIAA1718 blocks such neural differentiation. Chen *et al*. also identified FGF4 as the direct target of KIAA1718 that mediates the pro-neural differentiation effect[12].

NSCs have been successfully isolated from various regions of the embryonic central nervous system[13]. However, whether NSCs can be isolated from the embryonic peripheral nervous system has not been completely explored. Gu *et al*. attempted to answer this question by using fetal rat dorsal root ganglia[14]. The progenitor-like cells isolated from dorsal root ganglia resemble neural crest progenitors. Such cells formed neurospheres and generated

secondary and tertiary spheres by cloning assays, and could also give rise to neurofilament-expressing neurons and S100-expressing Schwann cell-like cells.

The fate of NSCs is largely based on the delicate balance between intrinsic and extrinsic factors. Extracellular matrix contains important components that regulate the different aspects of NSCs. An optimal biomaterial may capitalize on this information to produce scaffolds to support the survival and differentiation of NSCs for regenerative approaches. In a recent report, Li *et al*. examined three different biomaterials, chitosan membranes, collagen gels, and chitosan-collagen membranes, and compared the effects of those materials on the survival, proliferation and differentiation of rat NSCs. Among the three, chitosan-collagen membranes seemed to show the best effect in supporting the survival and neuronal production from rat NSCs[15].

It has long been debatable whether functional neurons may be derived from bone marrow-derived mesenchymal stem cells (BMSCs). The key issue has always been whether the neuron-like cells derived from MSCs demonstrate the electrophysiological properties of nerve cells [16–17]. Ge *et al*. tried to specifically direct BMSCs isolated from rhesus monkeys towards cholinergic neurons [18]. The authors compared four different conditions for neuronal differentiation, basal medium (bFGF and forskolin), basal medium+sonic hedgehog (SHH), basal medium +retinoic acid (RA), and basal medium+SHH+RA, and demonstrated that SHH+RA inducing group showed neuronal resting membrane potential and expressed high levels of synapsin and acetylcholine. However, in this study, the membrane potential was only tested by using a fluorescent dye DiBAC4. The whole cell clamp technique could have been used to more directly and accurately measure the membrane potential and analyze other electrophysiological properties of the resulting cells.

4 Research on mesenchymal stem cells

MSCs are stem cells that can self-renew and differentiate into mesodermal lineages including chondrocytes, adipocytes, and osteocytes. Due to their low immunogenicity and easy access from tissues such as bone marrow and adipose tissue, MSCs hold great promise in prospective clinical applications[19–20]. In the study reported by Qiu *et al*. [21], an alternative source material, placenta, was used to obtain MSCs. Human placenta-derived stem cells (hPDSCs) resemble MSCs in terms of surface marker expression and the differentiation potentials. hPDSCs possess a high proliferative ability and can be induced into type Ⅱ collagen-expressing chondrocytes. When seeded into collagen sponges, followed by introduction into nude mice, hPDSCs could develop into cartilage tissues *in vivo*, suggesting a potential utility in repair of cartilage defects.

It has been shown that bone marrow derived stem cells participate in the regeneration process after myocardium infarction, possibly by increasing growth factor production and/or decreasing proinflammatory cytokine production, rather than a direct replacement of the damaged cardiomyocytes [22–23]. Treatment with estrogen prior to MSC infusion leads to a better recovery from myocardium infarction, compared with non-treated MSC group. Estrogen treatment can significantly augment the production of vascular endothelial growth factor (VEGF) from MSCs. Hu *et al*. also found that estrogen treatment can markedly reduce the apoptosis and increase the viability and proliferation of MSCs [22], indicating that *ex vivo* treatment with estrogen may maximize the clinical potential of MSCs for repair of ischemic myocardium.

MSCs also have great potentials for treating disorders of the immune system. Shi's lab looked into the underlying mechanisms of the immunosuppressive effects of MSCs and found that priming with pro-inflammatory cytokines is necessary for MSCs to exert immunosuppressive functions [24]. Upon stimulation, MSCs secrete chemokines to attract immune cells into the proximity to MSCs, and suppress the immune functions through both cell-cell contact and soluble factors, with nitric oxide or indoleamine 2, 3-dioxygenase being key mediators in mouse MSCs and human MSCs, respectively.

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