

Stem cell research in China

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In the past 5 years, China has increased its efforts in the field of stem cell research and practice. Basic research mainly focuses on bone marrow and embryonic stem cells. Clinical applications of stem cells in the treatment of acute heart failure, acute liver failure and lower limb ischaemia have been reported by many hospitals. China enacted its 'Ethical Guidelines for Human Embryonic Stem Cell Research' in 2003. At present, China has the most liberal and favourable environments for human embryonic stem cell research.

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1. INTRODUCTION

Stem cells are defined as a cell population capable of self-renewal, proliferation and differentiation. Stem cells are usually classified into two different types during the development of the organism: embryonic stem cells (ESCs) and adult stem cells. ESCs are derived from the inner cell mass of blastocysts and continue their development by segregating into the hypoblast and epiblast. These layers further segregate into the pluripotent primary germ layers: ectoderm, mesoderm and endoderm. Adult stem cells are represented in many tissues of the adult organism. Their physiological function consists of renewing or restoration of the differentiated cells during the lifespan of the organism. The majority of regional adult stem cells differentiate into a limited number of cell types. For example, neural stem cells give rise to cells of the nervous system and haemopoietic stem cells to blood cells.

The beginnings of stem cell research in China may be traced back to 1963, 34 years before Dolly the sheep was introduced to the world, when the late embryologist Dizhou Tong transferred the DNA from a cell of a male Asian carp to an egg of a female Asian carp, and produced the world's first cloned fish (Tong *et al.* 1963). In previous decades, researchers had cloned micro-organisms and nematodes, as well as amphibians. But before Tong, nobody had ever managed to clone such a complex organism. To all appearances, the experiment was entirely successful. The cloned carp even sired a baby carp. After 10 years, Tong inserted the DNA of an Asian carp into an egg of a European crucian carp, a related species, and created the first interspecies clone (Tong *et al.* 1973). Based on this pioneering research, Chinese scientists developed fish-breeding techniques so powerful that the nation now produces more than half of the world's aquaculture harvest. But few, if any, Western scientists knew of

Tong's achievements, partly because his work was published in a Chinese journal, *Acta Zoologica Sinica*, which did not have an English-language abstract, a common problem in non-Western scientific periodicals. In any case, Tong performed his experiments not to study cloning *per se* but to investigate the interactions between DNA and the egg containing it. Unfortunately, Tong's pioneering work was interrupted prematurely by the Chinese Cultural Revolution.

Presently, no country is pursuing the field more aggressively than China. In China, research on both ESCs and adult stem cells is supported by governmental funds. Stem cell research fits the Chinese Ministry of Science and Technology's ambitious plans to vault the country to the top of the research ranks. China has pumped money into this area through multiple sources: cities, provincial governments and two special national research initiatives (863 and 973 plans). In this review, we will give a brief introduction to the stem cell research based on published reports both in English and Chinese.

2. HUMAN EMBRYONIC STEM CELLS

The Chinese government allows research on human embryos and cloning to continue for therapeutic purposes. In 2001, the two national committees on medical ethics and bioethics, the Beijing Ministry of Health Medical Ethics Committee and the Southern Chinese Human Genome Research Centre Ethical, Legal, and Social Issues Committee (ELSI), proposed ethical guidelines on human embryonic stem cell (hESC) research. The suggested regulations ban activities that most nations condemn such as reproductive human cloning and the buying and selling of human embryos for commercial purposes. They also proposed the establishment of a new organization to centralize the ethical management of stem cell research in China, a responsibility that is currently divided between the Ministry of Health, the Ministry of Science and Technology and the local ethics committees. Subsequently, the 'Four Nos' were proposed for

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the scientific community: a single-sentence directive promulgated by the Ministry of Health in November, 2002: 'Under no situation, under no circumstances, will human reproductive cloning experiments be 1) endorsed, 2) permitted, 3) supported, or 4) accepted'. On 24 December 2003, Ethical Guidelines for Research on Human Embryonic Stem Cells were enacted by the Ministry of Science and Technology and the Ministry of Health of China. The hESCs described in the guidelines include stem cells derived from donated human embryos, those originated from germ cells and those obtained from somatic cell nuclear transfer. It again clearly states that any research aiming at human reproductive cloning and hybridizing human germ cells with germ cells of any other species shall be prohibited.

He *et al.* (2002) established the first hESC lines in China. Fourteen human oocytes obtained from a volunteer were fertilized *in vitro*. Four of the morulae formed from the fertilized oocytes were frozen and five continued to develop into blastocysts. The pellucid zones of the five blastocysts were removed and their inner cell masses taken out and inoculated onto a feeder layer of mouse embryonic fibroblasts. The cell clones formed from the three surviving inner cell masses were selected, dispersed and inoculated onto feeder layers to produce more cell clones. When multiple clones were produced, they were dissociated with dispase into smaller cell masses, which were inoculated onto a new feeder layer again. Out of nine fertilized oocytes, five developed into blastocysts. Three hESC cell lines were established from the inner cell masses, namely CHE1, CHE2 and CHE3. These stem cells remained undifferentiated after subculturing for seven months. The surface markers of human stem cells, SSEA-4, SSEA-3, TRA-1-60 and GCTM-2, were positive at passages 25, 30 and 40 for CHE3, and at passages 21, 22 and 30 for CHE1 and CHE2. All cell lines stably retained a normal karyotype. After routine passage for five months, cells from three cell lines were inoculated onto the legs of severe combined immunodeficiency mice subcutaneously to observe teratoma formation. Six weeks after inoculation of the three cell lines, teratomas were formed in all mice. Histological examination revealed that they contained various tissues derived from all the three embryonic germ layers. Thus, He *et al.* (2002) established the first hESC lines in China.

The researchers led by Sheng of the Shanghai Second Medical University have reprogrammed human cells by fusing them with rabbit eggs emptied of their genetic material (Chen *et al.* 2003a). They extracted stem cells from the resulting embryos and derived ESCs by the transfer of human somatic nuclei into rabbit oocytes. The number of blastocysts that developed from the fused nuclear transfer was comparable among nuclear donors at the ages of 5, 42, 52 and 60 years, and nuclear transfer embryonic stem cells (ntES cells) were subsequently derived from each of the four age groups. These results suggest that human somatic nuclei can form ntES cells independent of the age of the donor. The derived ntES cells were human based on karyotype, isogenicity, *in situ* hybridization, polymerase chain reaction (PCR) and immunocytochemistry with probes

that distinguish between the various species. The ntES cells maintained the capability for sustained growth in an undifferentiated state and formed embryoid bodies, which, on further induction, gave rise to cell types such as neuron and muscle, as well as mixed cell populations that expressed markers representative of all the three germ layers. Thus, ntES cells derived from human somatic cells by nuclear transfer to rabbit eggs retain phenotypes similar to those of conventional hESCs, including the ability to undergo multilineage cellular differentiation. Sheng's group also established four hESC lines (Fang *et al.* 2005a).

A lot of effort has been put into the regulation of ESC proliferation and differentiation. *Nanog*, an NK-2-type homeodomain gene, has been proposed to play a key role in maintaining stem cell pluripotency. Pei and co-workers at the Institute of Pharmacology, Tsinghua University, showed that *Nanog* behaves as a transcription activator with two unusually strong activation domains embedded in its C-terminus. They identified two transactivators by employing the Gal4 DNA-binding domain fusion and reporter system and named them WR and CD2. CD2 contains no obvious structural motif, whereas the WR or Trp repeat contains 10 pentapeptide repeats starting with a Trp in each unit. Deletion of both WR and CD2 from *Nanog* completely eliminated its transactivation function (Pan & Pei 2004). They also identified and characterized (195)RKRKR as the nuclear localization signal responsible for *Oct4* localization and required for the transactivation of its target genes in ESC (Pan *et al.* 2004). Xu *et al.* (2004) at the Shanghai Second Medical University described a novel murine ubiquitin ligase, *Wwp2*, that specifically interacts with Oct-4 and promotes its ubiquitination both *in vivo* and *in vitro*. Remarkably, the expression of a catalytically inactive point mutant of *Wwp2* abolished Oct-4 ubiquitination. Moreover, *Wwp2* promoted Oct-4 degradation in the presence of overexpressed ubiquitin. Fusion of a single ubiquitin to Oct-4 inactivated it transcriptionally in a heterologous Oct-4-driven reporter system. Furthermore, overexpression of *Wwp2* in ESCs significantly reduced Oct-4 transcriptional activities. Collectively, they demonstrate for the first time that Oct-4 can be post-translationally modified by ubiquitination and that this modification dramatically suppresses its transcriptional activity. Their results open up a new avenue to understanding how Oct-4 defines the fate of ESCs.

3. PLURIPOTENT STEM CELL

Although the potential applications of hESCs and therapeutic cloning hold promise of medical benefits, these technologies have posed profound ethical issues because they necessitate the destruction of human embryos. We should always keep in mind that science and technology can never be independent of the criterion of morality, since technology exists for man. Hence, a fundamental point in the issues of embryonic stem cells is the concept of the moral status of human embryos. A lot of people have held that human life begins at the moment of conception and therefore, have defended the dignity, inviolable right to life and

integrity of human embryos. Some governments have opposed limitations on hESC research. Therefore, it is of great importance for scientists to find adult stem cells that have a similar differentiation potential to embryonic stem cells.

Researchers led by Zhao at the Chinese Academy of Medical Sciences reported that a cell population derived from human foetal bone marrow, termed Flk1⁺CD31⁻CD34⁻ stem cells, could differentiate, not only into osteogenic, adipogenic and endothelial lineages, but also hepatocyte-like, neural and erythroid cells at the single-cell level (Fang *et al.* 2003, 2004).

Zhao *et al.* used cells from a single colony, which precluded the possibility of contamination by different stem cells. Based on their research, they suppose that there is a subfraction of the adult stem cell population which exists in a number of tissues beyond embryo development. These stem cells can form tissues of different germ layer lineages, differing from common adult stem cells that only form tissues within a particular germ layer lineage. However, they differ from ESCs in that they gradually lose some differentiation potential during gestation and adopt special phenotypes or molecular markers once within a certain kind of tissue. They remain in some/all tissues and organs during gestation and can give rise to different kinds of pluripotent stem cells, contributing to self-repair and self-renewal. They can provide cells not only for the damaged tissues in which they reside, but also for remote tissues by migration triggered by proinflammatory cytokines or growth factors.

To test the hypothesis that post-embryonic subtotipotent stem cells exist in most human tissues, numerous experiments have been carried out in the lab. Zhao and co-workers have chosen foetuses as a source of adult stem cells. They believe that the foetus may contain the most primitive stem cells and will make the isolation of cells easier. In China, given that research on spontaneously aborted human foetuses is lawful upon receiving written consent from the donor, a lot of researchers are using human foetuses as a source of cells (Tang 2003).

They found that Flk1⁺CD31⁻CD34⁻ cells isolated from foetal bone marrow have characteristics of haemangioblasts, i.e. progenitors of endothelial and haemopoietic cells (Guo *et al.* 2003). They showed that on extra-cellular matrix (ECM) gel, Flk1⁺CD31⁻CD34⁻ cells could grow into a vascular structure that was positive for CD31 and vWF. When the angiogenesis inhibitor suramin was added, formation of a vascular structure was blocked. In addition, Flk1⁺CD31⁻CD34⁻ cells cultured in haemopoietic conditions could differentiate into haemopoietic cells which expressed GATA-1, -2, gamma- and beta-globin genes. After being replated in methylcellulose medium, they formed typical erythroid colonies. The results suggested that these Flk1⁺CD31⁻CD34⁻ cells bear characteristics of haemangioblasts after the embryo stage. These findings were further confirmed in chronic myelogenous leukaemia (CML; Fang *et al.* 2005b). They isolated Flk1⁺ cells carrying the *BCR/ABL* fusion gene from the bone marrow of 17 Philadelphia chromosome-positive (Ph⁺) patients with CML and found that they could differentiate into malignant

blood cells and phenotypically defined endothelial cells at the single-cell level. These findings provide direct evidence, for the first time, that rearrangement of the *BCR/ABL* gene might happen at or even before the level of haemangioblastic progenitor cells, thus resulting in the detection of the *BCR/ABL* fusion gene in both blood and endothelial cells.

The potential use of Flk1⁺CD34⁻ stem cells in tissue regeneration was demonstrated in several models. First, when fluorescence-labelled Flk1⁺CD34⁻ stem cells of BALB/c mice (H-2K^d, white) were transplanted into lethally irradiated C57BL/6 mice (H-2K^b, black), donor cells could migrate and take residency at the skin, which was confirmed by Y chromosome-specific PCR and Southern blot. The recipient mice grew white hairs after approximately 40 days. Immunocytochemistry staining and RT-PCR demonstrated that skin tissue within the white hair regions was largely composed of donor-derived H-2K^d cells, including stem cells and committed cells. Furthermore, most skin cells cultured from white hair skin originated from the donor. Thus, these findings provide direct evidence that bone marrow-derived cells can give rise to functional skin cells and regenerate skin tissue (Deng *et al.* 2005a,b). Second, Flk1⁺CD34⁻ stem cells from adipose tissue were shown to have characteristics of endothelial progenitor cells. *In vitro*, these cells expressed endothelial markers when cultured with vascular endothelial growth factor (VEGF). *In vivo*, these cells can differentiate into endothelial cells that contributed to neoangiogenesis in the hind limb ischaemia models, suggesting they may be a potential source of endothelial cells for cellular proangiogenic therapies (Cao *et al.* 2005).

Zhao and co-workers also demonstrated that Flk1⁺CD34⁻ stem cells can modulate immune function. Consistent with the finding that Flk1⁺CD34⁻ stem cells have the characteristics of haemangioblasts, they showed that these cells could induce stable mixed chimerism and donor-specific graft tolerance when they were transplanted into lethally irradiated mice. FACS analysis revealed that more than 5% of donor-derived CD3⁺ cells were detected in splenocytes of recipient mice. Long-term survival of donor-type skin grafts was observed. Mixed lymphocyte reaction (MLR) and mitogen proliferative assays showed that the recipient mice had low immune response to donor cells but retained a normal ConA-induced proliferative response compared with normal mice (Deng *et al.* 2004). Further studies showed that Flk1⁺CD34⁻ stem cells could inhibit the upregulation of CD1a, CD40, CD80, CD86 and HLA-DR during dendritic cell differentiation and prevent an increase of CD40, CD86 and CD83 expression during dendritic cell maturation. Flk1⁺CD34⁻ stem cells interfered with endocytosis of dendritic cells and decreased their capacity to secrete IL-12 and activate alloreactive T cells (Zhang *et al.* 2004). More importantly, Flk1⁺CD34⁻ stem cells from BALB/c mice had inhibitory effects on BXS mice T-lymphocyte proliferation. Furthermore, Flk1⁺CD34⁻ stem cells had inhibitory effects on the proliferation, activation and IgG secretion of B lymphocytes. In addition, BALB/c Flk1⁺CD34⁻ stem cells had an enhancing effect on CD40 expression and inhibitory effects on CD40 ligand (CD40L)

ectopic hyperexpression on B cells from BXSB mice. In further studies, they found that transplantation of Flk1⁺CD34⁻ stem cells from BALB/c mice alleviated the symptoms of BXSB mice. As the BXSB mouse is considered an experimental model for human systemic lupus erythematosus, these findings may have important implications (Deng *et al.* 2005a,b).

Li's group at the Peking University Stem Cell Centre observed that cells isolated from human gonadal ridges and mesenteries expressed pluripotent markers and formed embryonic bodies. However, these cells did not proliferate well and tended to differentiate spontaneously into neuron-like cells under their culture conditions (Pan *et al.* 2005). It is obvious that more effort is needed to reproduce the perfect growth conditions for embryonic gonadal cells in order to maintain them long term in culture. They also isolated mesenchymal stem cells (MSC) from umbilical-cord blood (UCB). They showed that MSC-like cells could be isolated and expanded from 16- to 26-week foetal blood, but were not acquired efficiently from full-term infants' UCB. MSC-like cells shared a similar phenotype with bone-marrow MSC: CD34⁻CD45⁻CD44⁺CD71⁺CD90⁺CD105⁺. They could be induced to differentiate into osteogenic, adipogenic and neural lineage cells. Single-cell clones also showed similar phenotypes and differentiation ability. These results suggest that MSC are abundant in early foetal blood but not in term UCB (Yu *et al.* 2004).

4. APPLICATION OF ADULT STEM CELLS

The most significant achievements made in China can be recognised by the quick transfer of the basic research to clinical application.

Zhao's group are in collaboration with the National Institute for the Control of Pharmaceutical and Biological Products. Based on the principles of safety, reliability, stability and controllability, they collectively worked out a highly standard and stringent procedure for Flk1⁺CD31⁻CD34⁻ stem cell culture and assessment. Preclinical studies on monkeys have showed that this approach could greatly promote the survival of newly transplanted stem cells and reconstitution of the haemopoietic system and significantly reduce the degree of graft versus host disease. As a 'new cell drug', the Flk1⁺CD31⁻CD34⁻ stem cell clinical protocol won approval from the State Food and Drug Administration of China in 2004. Now Zhao's group is running China's first officially approved stem cell therapy for patients with leukaemia and other severe diseases. They showed that cotransplantation of HLA-identical sibling culture-expanded Flk1⁺CD31⁻CD34⁻ stem cells with an HLA-identical sibling hematopoietic stem cell (HSC) transplant is feasible and seems to be safe, without immediate or late stem cell-associated toxicities. The number of patients included in phase I clinical trials did not allow a demonstration of the effectiveness of the treatment. However, the result of the present study is encouraging and phase II clinical trials will soon be started.

In acute myocardial infarction, the flow of blood from a blood vessel in the heart is blocked, the cardiac muscle receives insufficient oxygen and the heart

tissue dies. In many cases, supply of blood to the deadened portion of the heart can be restored via the so-called balloon technique. But the heart suffers permanent damage, primarily to the left ventricle. Researchers in Nanjing have tested the administration of bone marrow stem cells in patients stricken with acute myocardial infarction. Sixty-nine patients who underwent primary percutaneous coronary intervention within 12 hours of the onset of acute myocardial infarction were randomized to receive intracoronary injection of autologous bone-marrow MSCs or saline. Several imaging techniques demonstrated that bone-marrow MSCs significantly improved left ventricular function. For example, the proportion with a functional defect decreased significantly in the MSC group after three months. Wall movement velocity over the infarcted region increased significantly in the MSC group, but not in the control. The left ventricular ejection fraction in the MSC group increased significantly three months after transplantation compared with pre-implantation and with that of the control group at three months post-injection. Single-photon emission-computed tomography scan results showed that the perfusion defect had improved significantly in the MSC group at the three-month follow-up compared with that of the control group (Chen *et al.* 2004). Similar MSC therapy for heart infarction was carried out in other hospitals (Chen *et al.* 2003b; Ruan *et al.* 2005; Zhang *et al.* 2005).

Allogeneic liver transplantation remains the only effective treatment available to patients with liver failure. Owing to a serious shortage of liver donors, an alternative therapeutic approach is urgently needed. Transplantation of hepatocytes derived from adult or foetal livers is not a candidate for the alternative treatment because the source of such cells is limited to human liver at present. Recently, extrahepatic sources of cells have been explored for use in cell therapy. Yao *et al.* (2005) reported the treatment of patients with liver failure by the transplantation of autologous bone-marrow stem cells. Bone marrow was harvested (30–50 ml) from patients in the transplant group and infused into the liver of patients via the hepatic artery. At different time points (one, two, four or eight weeks post-transplantation), alanine aminotransferase (ALT), total bilirubin (TBIL), direct bilirubin (DBIL), albumin (ALB) and prothrombin time activity (PTA) were measured, and the survival rate and improvement of symptoms recorded. After transplantation of bone-marrow stem cells, the liver function of patients improved. Eight weeks after transplantation, ALT reduced from 181.71 to 72.1 $\mu\text{mol l}^{-1}$, TBIL from 153.1 to 80.2 $\mu\text{mol l}^{-1}$, DBIL from 74.1 to 40.5 $\mu\text{mol l}^{-1}$, ALB increased from 26.5 to 31.5 $\mu\text{mol l}^{-1}$ and PTA from 28.23 to 50.1%, respectively. The survival rate of the transplant group was higher than that of the control. Eight weeks after transplantation, ascites decreased in 10 cases (50%), appetite improved in 15 cases (75%) and abdomen distention ameliorated in 9 cases (45%). No serious side effects were observed in 20 patients with bone-marrow stem cell transplantation (Yao *et al.* 2005).

Diabetes is a common chronic disease with significant morbidity and mortality. One devastating complication of diabetes is peripheral arterial disease

including critical limb ischaemia, which may result in limb loss. There is no available permanent cure for diabetic limb ischaemia at present. In response to tissue injury and remodelling, neovascularization usually occurs via the proliferation and migration of endothelial cells from pre-existing vasculature. The endothelial progenitor cells (EPCs) resident within the bone marrow and peripheral blood can also contribute to injury- and pathology-induced neovascularization. Therefore, therapeutic angiogenesis induced by transplantation of functional EPCs into ischaemic tissues may represent a novel approach for diabetic patients with limb ischaemia. Yang and co-workers reported a simple and effective therapeutic approach for diabetic limb ischaemia by autologous transplantation of granulocyte-macrophage colony-stimulating factor (G-CSF)-mobilized peripheral blood stem cells (PBSCs). In total, 62 patients with 34 cases of diabetic foot and 28 cases of various lower extremity ischaemic disorders received recombinant human G-CSF at $450\text{--}600\ \mu\text{g d}^{-1}$ by hypodermic injection for 5 days to mobilize stem cells. On the 6th day, PBSCs were collected. The PBSCs were injected into the ischaemic lower extremity and foot intramuscularly. The clinical and laboratory findings were monitored from the first day to the twenty-fourth week. In 62 patients with PBSC transplantation, 54 cases (87.1%) were found to be free of severe pain after 7 to 30 days. An improvement in foot coolness and ulceration was observed in 56 patients (90.3%) after 7 to 30 days and in 16 cases (40.0%) after 4 to 16 weeks, respectively. Ankle/brachial index increased in 12 cases (34.3%) and transcutaneous PO_2 (TcPO_2) in 26 cases (42.3%). Digital subtraction angiographic scores indicated the formation of new collateral vessels. Adverse effects were only observed in two patients during the process of stem cell mobilization (Yang et al. 2005a). Other researchers have also reported their findings (Huang et al. 2004; Yang et al. 2005b).

Spinal cord injury (SCI)-induced paralysis is related to the fact that axons in adult central nervous system (CNS) do not regenerate after injury. Several factors may account for the normal regenerative failure seen in the adult CNS. Implantation of various types of tissue, such as peripheral nerve grafts, ESCs, macrophages and olfactory ensheathing cells (OECs), into and around the site of SCI have been shown to contribute to spontaneous axonal regeneration following injury; implanted OECs seem to have the most promising effect on it (Li et al. 1997). The olfactory system is unique in that it supports continuous growth of axons from the olfactory epithelium into the CNS throughout the lifetime of the individual. The olfactory system's capacity for axonal extension and target-specific synaptic interaction has been attributed to the growth-promoting function of ensheathing cells. Huang et al. (2003) reported the restoration of function after SCI in patients of different ages who underwent intraspinal transplantation of OECs. In their study, 171 SCI patients were included. In all these SCI patients, the lesions were injected with OECs at the time of operation. Spinal cord function was assessed based on the American Spinal Injury Association (ASIA) classification system before and two-to-eight weeks after OEC transplantation. After surgery, the motor

scores increased by 5.2 ± 4.8 , 8.6 ± 8.0 , 8.3 ± 8.8 , 5.7 ± 7.3 and 8.2 ± 7.6 in five age groups; light touch scores increased by 13.9 ± 8.1 , 15.5 ± 14.3 , 12.0 ± 14.4 , 14.1 ± 18.5 and 24.8 ± 25.3 ; and pin prick scores increased by 11.1 ± 7.9 , 17.2 ± 14.3 , 13.2 ± 11.8 , 13.6 ± 13.9 and 25.4 ± 24.3 , respectively. OEC transplantation can improve the neurological function of spinal cord of SCI patients regardless of their ages. Further research into the long-term outcomes of the treatment will be required (Huang et al. 2003).

5. CONCLUSION

Stem cell research in China is attracting more and more attention from around the world. Many of the top labs are planning to submit their most promising results to international research journals. This may consolidate the number of labs working in the field as funding institutions concentrate their resources on the few labs that produce results. While the Chinese government has issued guidelines for hESC research, some scientists and ethicists also expect guidelines for the clinical use of adult stem cells. However, no laws or enforcement can be realistically expected in the short term. Presently, only Zhao's group at the Chinese Academy of Medical Sciences and Peking Union Medical College, China, has been granted phase I clinical trial approval for the use of MSCs in cotransplantation of haemopoietic stem cells for leukaemia patients.

REFERENCES

- Cao, Y., Sun, Z., Liao, L. M., Meng, Y., Han, Q. & Zhao, R. C. 2005 Human adipose tissue-derived stem cells differentiate into endothelial cells *in vitro* and improve postnatal neovascularization *in vivo*. *Biophys. Biochem. Res. Commun.* **332**, 370–379.
- Chen, Y. et al. 2003a Embryonic stem cells generated by nuclear transfer of human somatic nuclei into rabbit oocytes. *Cell Res.* **13**, 251–263. (doi:10.1038/sj.cr.7290170)
- Chen, Y. X., Ou, R. L., Zhong, X. Y., Xu, X., Zhao, H. Y., Guang, H. H., Lu, Y. & Han, Z. C. 2003b Repair of infarcted myocardium by intramyocardial transplantation of mobilized autologous bone marrow stem cells in humans. *Chin. J. Pathophysiol.* **19**, 452–454.
- Chen, S. L. et al. 2004 Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction. *Am. J. Cardiol.* **94**, 92–95. (doi:10.1016/j.amjcard.2004.03.034)
- Deng, W., Han, Q., Liao, L., Li, C., Ge, W., Zhao, Z., You, S., Deng, H. & Zhao, R. C. 2004 Allogeneic bone marrow-derived $\text{Flk1}^+\text{Sca1}^-$ mesenchymal stem cells leads to stable mixed chimerism and donor-specific tolerance. *Exp. Hematol.* **32**, 861–867. (doi:10.1016/j.exphem.2004.06.009)
- Deng, W. M. et al. 2005a Engrafted bone marrow-derived Flk1^+ mesenchymal stem cells regenerate skin tissue. *Tissue Eng.* **11**, 110–119. (doi:10.1089/ten.2005.11.110)
- Deng, W. M., Han, Q., Liao, L. M., You, S., Deng, H. & Zhao, R. C. 2005b Effects of allogeneic bone marrow-derived mesenchymal stem cells on T and B lymphocytes from BXS mice. *DNA Cell Biol.* **24**, 458–463. (doi:10.1089/dna.2005.24.458)
- Fang, B., Shi, M., Liao, L., Yang, S., Liu, Y. & Zhao, R. C. 2003 Multiorgan engraftment and multilineage differentiation by human fetal bone marrow $\text{Flk1}^+\text{CD31}^-\text{CD34}^-$

- progenitors. *J. Hematother. Stem Cell Res.* **12**, 603–613. (doi:10.1089/15258160360732632)
- Fang, B., Liao, L., Shi, M., Yang, S. & Zhao, R. C. 2004 Multipotency of Flk1⁺CD34⁻ progenitors derived from human fetal bone marrow. *J. Lab. Clin. Med.* **143**, 230–240. (doi:10.1016/j.lab.2003.11.008)
- Fang, Z. F., Jin, F., Gai, H., Chen, Y., Wu, L., Liu, A. L. & Sheng, H. Z. 2005a Human embryonic stem cell lines derived from the Chinese population. *Cell Res.* **15**, 394–400. (doi:10.1038/sj.cr.7290307)
- Fang, B., Zheng, C., Liao, L., Han, Q., Sun, Z., Jiang, X. & Zhao, R. C. 2005b Identification of human chronic myelogenous leukemia progenitor cells with hemangioblastic characteristics. *Blood* **105**, 2733–2740. (doi:10.1182/blood-2004-07-2514)
- Guo, H., Fang, B., Liao, L., Zhao, Z., Liu, J., Chen, H., Hsu, S. H., Cui, Q. & Zhao, R. C. 2003 Hemangioblastic characteristics of fetal bone marrow-derived Flk1⁺CD31⁻CD34⁻ cells. *Exp. Hematol.* **31**, 650–658. (doi:10.1016/S0301-472X(03)00087-0)
- He, Z., Huang, S., Li, Y. & Zhang, Q. 2002 Human embryonic stem cell lines preliminarily established in China. *Zhonghua Yi Xue Za Zhi.* **82**, 1314–1318.
- Huang, H. *et al.* 2003 Influence of patients' age on functional recovery after transplantation of olfactory ensheathing cells into injured spinal cord injury. *Chin. Med. J.* **116**, 1488–1491.
- Huang, P. P., Li, S. Z., Han, M. Z., Xiao, Z. J., Yang, R. C., Qiu, L. G. & Han, Z. C. 2004 Autologous transplantation of peripheral blood stem cells as an effective therapeutic approach for severe arteriosclerosis obliterans of lower extremities. *Thromb. Haemost.* **91**, 606–609.
- Li, Y., Field, P. M. & Raisman, G. 1997 Repair of adult rat corticospinal tract by transplants of olfactory ensheathing cells. *Science* **277**, 2000–2002. (doi:10.1126/science.277.5334.2000)
- Pan, G. & Pei, D. 2004 The stem cell pluripotency factor NANOG activates transcription with two unusually potent subdomains at its C terminus. *Biol. Chem.* **280**, 1401–1407. (doi:10.1074/jbc.M407847200)
- Pan, G., Qin, B., Liu, N., Scholer, H. R. & Pei, D. 2004 Identification of a nuclear localization signal in OCT4 and generation of a dominant negative mutant by its ablation. *J. Biol. Chem.* **279**, 37 013–37 020. (doi:10.1074/jbc.M405117200)
- Pan, Y., Chen, X., Wang, S., Yang, S., Bai, X., Chi, X., Li, K., Lui, B. & Li, L. 2005 *In vitro* neuronal differentiation of cultured human embryonic germ cells. *Biochem. Biophys. Res. Commun.* **327**, 548–556. (doi:10.1016/j.bbrc.2004.11.168)
- Ruan, W., Pan, C. Z., Huang, G. Q., Li, Y. L., Ge, J. B. & Shu, X. H. 2005 Assessment of left ventricular segmental function after autologous bone marrow stem cells transplantation in patients with acute myocardial infarction by tissue tracking and strain imaging. *Chin. Med. J.* **118**, 1175–1181.
- Tang, P. H. 2003 Current basic research of hematopoietic stem cells in China and comments on stem cell plasticity. *Zhongguo Shi Yan Xue Ye Xue Za Zhi.* **11**, 1–6.
- Tong, D. Z., Wu, S. Q., Ye, Y. F., Yang, S. Y., Du, M. & Lu, D. Y. 1963 Nuclear transfer in fishes. *Sci. Bull.* **7**, 60–61.
- Tong, D. Z., Ye, Y. H., Lu, D. Y., Tong, S. M. & Du, M. 1973 Transplantation of nuclei between two subfamilies of teleosts (Goldfish-domesticated carassius and Chinese bitterling *Rhodeus sinensis*). *Acta Zool. Sin.* **19**, 201–207.
- Xu, H. M. *et al.* 2004 Wwp2, an E3 ubiquitin ligase that targets transcription factor Oct-4 for ubiquitination. *J. Biol. Chem.* **279**, 23 495–23 503. (doi:10.1074/jbc.M400516200)
- Yang, X. F., Wu, Y. X., Wang, H. M., Xu, Y. F., Lu, X., Zhang, Y. B., Wang, F. & Zhang, Y. 2005a Autologous peripheral blood stem cells transplantation in treatment of 62 cases of lower extremity ischemic disorder. *Zhonghua Nei Ke Za Zhi.* **44**, 95–98.
- Yang, B. H., Qin, J. H., Zhu, L. Q., Chen, L., Lu, H., Liu, F. T. & Li, Y. S. 2005b Treatment of lower limb ischemia with combination of traditional Chinese medicine and transplantation of autologous bone marrow mononuclear cells: a report of 23 cases. *Zhong Xi Yi Jie He Xue Bao* **3**, 28–30.
- Yao, P., Hu, D. R., Wang, S., Wen, W., Zhou, Y. R. & Gong, L. J. 2005 Treatment of chronic hepatic failure with autologous bone marrow stem cells transplantation. *Chin. Hepatol.* **10**, 171–173.
- Yu, M., Xiao, Z., Shen, L. & Li, L. 2004 Mid-trimester fetal blood-derived adherent cells share characteristics similar to mesenchymal stem cells but full-term umbilical cord blood does not. *Br. J. Haematol.* **124**, 666–675. (doi:10.1111/j.1365-2141.2004.04826.x)
- Zhang, W., Ge, W., Li, C., You, S., Liao, L., Han, Q., Deng, W. & Zhao, R. C. 2004 Effects of mesenchymal stem cells on differentiation, maturation, and function of human monocyte-derived dendritic cells. *Stem Cells Dev.* **13**, 263–271. (doi:10.1089/154732804323099190)
- Zhang, M., Li, Z., Cui, L., Jin, Y., Yuan, L., Zhang, W. & Zhao, R. C. 2005 The safety of autologous peripheral blood stem cell transplantation by intracoronary infusion in patients with acute myocardial infarction. *S. China J. Cardiol.* **6**, 49–52.