Osteoarthritis and Cartilage 25 (2017) 616–624

Osteoarthritis and Cartilage

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Review

The potential of induced pluripotent stem cells as a tool to study skeletal dysplasias and cartilage-related pathologic conditions

H. Liu †, L. Yang †, F.F. Yu †, S. Wang †, C. Wu †, C. Qu ‡, M.J. Lammi † ‡ **, X. Guo † *

† School of Public Health, Health Science Center, Xi'an Jiaotong University, Key Laboratory of Trace Elements and Endemic Diseases, National Health and Family Planning Commission of PR China, Xi'an, Shaanxi 710061, PR China
‡ Department of Integrative Medical Biology, Umeå University, Umeå, Sweden

ARTICLE INFO

Article history:
Received 8 April 2016
Accepted 28 November 2016

Keywords:
Osteoarthritis
Induced pluripotent stem cells
Regenerative medicine
Drug screening

SUMMARY

The development of induced pluripotent stem cells (iPSCs) technology has opened up new horizons for development of new research tools especially for skeletal dysplasias, which often lack human disease models. Regenerative medicine and tissue engineering could be the next areas to benefit from refinement of iPSC methods to repair focal cartilage defects, while applications for osteoarthritis (OA) and drug screening have evolved rather slowly. Although the advances in iPSC research of skeletal dysplasias and repair of focal cartilage lesions are not directly relevant to OA, they can be considered to pave the way to future prospects and solutions to OA research, too. The same problems which face the present cell-based treatments of cartilage injuries concern also the iPSC-based ones. However, established iPSC lines, which have no genomic aberrations and which efficiently differentiate into extracellular matrix secreting chondrocytes, could be an invaluable cell source for cell transplantations in the future. The safety issues concerning the recipient risks of teratoma formation and immune response still have to be solved before the potential use of iPSCs in cartilage repair of focal cartilage defects and OA.

Introduction

Clinical problems related to articular cartilage diseases extend from genetic defects causing a number of chondrodysplasias to articular cartilage defects generally caused by traumatic injuries or a progressive degenerative disease called osteoarthritis (OA). The understanding of molecular mechanisms behind the chondrodysplasias is often difficult due to, for instance, the lethality of the disease, and new methods are needed to recapitulate the pathological features. Clinically, the treatment of the focal lesions of articular cartilage is somewhat less challenging than treating the OA, which is a systemic pathologic condition[1–3]. The etiology of the OA is multifactorial, and both the genetic and the environmental factors contribute to a risk of the disease. However, it is not a single disease. Metabolic, inflammatory or overloading factors (e.g., obesity or joint malalignment) represent their own mechanisms for the disease. Ageing itself can lead to the OA symptoms due to changes in the structure and function of the tissues, for instance, due to development of biomechanically less competent cartilage *via* cleavage of aggrecan monomers and formation of cross-links generating advanced glycation end-products.

It is also important to notice that besides articular cartilage the joint diseases involve the whole joint, including subchondral plate, bone, menisci, synovial membrane, ligaments and muscles[4]. Therefore, *in vitro* culture models, which focus only on cartilage, are inadequate to evaluate the whole nature and course of the disease. Yet they can be considered very valuable for studies on human early OA. One advantage of studies using chondrocytes or cartilage is that they lack the heterogeneity and, thus, the complexity of data interpretation compared with systems containing multiple cell types.

The clinical manifestations of the joint diseases are often associated with an obvious dysfunction of the joint, as well as symptoms and signs of inflammation, including chronic pain, stiffness, joint deformities and a loss of mobility[5,6]. They have a significant influence on the daily activities, and combined with the great health and economic burden, it is a major social issue[7]. The articular

* Address correspondence and reprint requests to: X. Guo, School of Public Health, Health Science Center of Xi'an Jiaotong University, No. 76 Yan Ta West Road, Xi'an, Shaanxi 710061. PR China. Fax: 86-029-82655032.
** Address correspondence and reprint requests to: M.J. Lammi, Department of Integrative Medical Biology, Umeå University, 901 87 Umeå, Sweden.
E-mail addresses: mikko.lammi@umu.se (M.J. Lammi), guox@mail.xjtu.edu.cn (X. Guo).

http://dx.doi.org/10.1016/j.joca.2016.11.015
1063-4584/

Table I
Main characteristics of ESCs and iPSCs

	Advantages	Disadvantages
Embryonic stem cells	Unlimited self-renewal Unlimited proliferation Pluripotent Potentially unlimited supply	Ethical concerns Religion controversy Tumorigenic potential Difficulty *in vitro* work Difficulty in guiding and controlling specific differentiation
Induced pluripotent stem cells	Autologous origin Extensive sources Unlimited self-renewal Unlimited proliferation Pluripotent No ethical issues No immune rejection (?)	Security Tumorigenic potential Inefficiency Instability Unclear mechanism Difficulty in guiding and controlling specific differentiation

cartilage has a limited capacity for self-repair due to its absence of vascularity, and currently there are generally no effective therapies for the injured articular cartilage[8]. The current treatments are focused on relieving the symptoms, improving the function and reducing the disability by a use of pharmacological interventions and non-pharmacological measures (exercise, weight loss, acupuncture, electrotherapy and surgical procedures)[9]. Therefore, it is urgent to develop new strategies to study the mechanisms of the disease and to find new, more effective treatments for it.

Recent advances in regenerative medicine suggest that stem cell therapies can be promising approaches. Embryonic stem cells (ESCs) have high proliferation potential, and they can be expanded almost unlimitedly without the cells undergoing to senescence. However, the derivation of the pluripotent stem cells from the early embryos raises both ethical and immune system-related limitations for further research, and especially for the clinical applications[10]. However, a wider availability of the pluripotent cell became possible when technique for induced pluripotent stem cells (iPSCs) reprogramming from somatic cells using specific transcription factors (TFs) was introduced[11,12]. The iPSCs are similar to the ESCs in their cell morphology, surface markers, pluripotency, proliferation capability, gene expression and many other aspects (Table I)[13–15]. In addition, the iPSCs do not involve such ethical, political and religious issues as the ESCs, and can apparently avoid the risk of immune rejection[16,17]. It has been demonstrated that the iPSCs have the potential to be successfully induced into chondrocytes[14,18–24] and osteoblasts[25,26], and be used to develop cellular models of the osteodegenerative diseases[20]. Therefore, the iPSCs are considered as a new promising tool to investigate the disease mechanisms and clinical applications, such as disease modeling, regenerative medicine and drug screening of the OA[26].

Generation of iPSCs

The iPSCs are pluripotent cells, which are reprogrammed to an embryonic-like pluripotent state from the somatic cells *in vitro* by the introduction and forced expression of four defined genes[11,12]. The development of the reprogramming technology has remarkably changed the understanding on regeneration capacity of the somatic cells, and together with advancements in CRISPR/Cas9 gene editing technology has provided efficient new tools and disease models for biomedical research[27,28].

In principle, each actively dividing somatic cell type can be used for the reprogramming, and the iPSCs have been generated from numerous different somatic cell types[11,12,26,29–43]. Due to reasons, such as convenience, efficiency and safety, the mostly used donor cell type have mainly been the skin fibroblast. Yet, small biopsies have to taken to get fibroblasts or skin keratinocytes. Therefore, the easy accessibility and low sampling invasiveness of blood cells and hair follicle keratinocytes make them somewhat more attractive cellular sources also when it concerns safety issues.

The efficiencies of reprogramming vary to some extent depending on the somatic cell sources used for iPSCs generation, as indicated in Table II. Although a good efficiency gives higher yield of iPSC colonies and is primarily useful for the purpose of studying reprogramming and its mechanism, most often it is not the major concern. Especially for clinical purposes and development of disease models, the quality and safety of the iPSC lines are much more important.

Initially, the iPSCs were generated using either retrovirus[11] or lentivirus[12] (Fig. 1). However, due to the random integration of transgenes, this approach increases the risk of insertional mutagenesis and tumor formation[10,44]. Although the integrating systems are efficient tools, the non-integrating methods are nowadays the mostly preferred present selection for the iPSCs generation. They have been successfully tried although with varying degrees of efficiency. Non-integrating viral vectors include adenovirus[45] and Sendai virus[46]. The former has a low efficiency and kinetics, while the Sendai virus (which is a non-integrating RNA virus) is efficiently expressed. Many other ways to generate integration-free iPSCs have been reported, including plasmids[47], recombinant proteins[48,49], synthesized mRNAs[50], and Cre/loxP system[51], episomal vectors[52], and piggyBac transposons[53,54]. In addition, small molecule combinations make full chemical induction of iPSCs possible[55,56]. The

Table II
The reprogramming technologies and efficiencies of different somatic cell sources used for iPSCs generation

Cell sources	Reprogramming factors	Reprogramming technologies	Efficiency (%)	References
Fibroblasts	OSKM	Retrovirus	0.01	11
Keratinocytes	OSKMN	Lentivirus	0.01–0.03	29
Cord blood cells	OSNL	Lentivirus	0.01–0.03	31
Blood cells	OSNL	Lentivirus	0.01–0.03	32
Adipose stem cells	OSKM	Lentivirus	0.01–0.03	33
Neural stem cells	O/OK	Retrovirus	0.004–0.006	34
Melanocytes	OSKM/OKS	Lentivirus	0.05/0.01	35
Hepatocytes	OSKM	Retrovirus	0.1–0.2	36
Circulating T Cells	OSKM	Sendai virus	0.1	37
Mesangial cells	OSKM	Retrovirus	NG	39
Urine cells	OSKM	Retrovirus	0.1–4.0	40
Synovial cells	OSKM	Retrovirus	~0.007–0.01	41
Pancreatic islet beta cells	OSKM	Lentivirus	0.0001	42
Chondrocytes	OSKM	Lentivirus	Not determined	10
OA chondrocytes/fibroblasts	OSKML	mRNA	0.1/2.0	43

Reprogramming factor: O, Oct4; S, Sox2; K, Klf4; M, c-Myc; N, Nanog; L, Lin28.