Guest editorial

Shinya Yamanaka's 2012 Nobel Prize and the radical change in orthopedic strategy thanks to his discovery of iPS cells

Professor Shinya Yamanaka of Kyoto University won the 2012 Nobel Prize in Physiology or Medicine. He discovered that mature cells can be reprogrammed to induce pluripotent stem cells (iPSCs), which can differentiate into any type of cell by introducing 4 reprogramming factors (c-Myc, Klf4, Oct3/4, and Sox2). Dr Yamanaka started his medical career as an orthopedic surgeon in 1987 after graduating from Kobe university. The inspiration for his choice of study and career as an orthopedic surgeon came from numerous fractures and dislocations experienced by both himself and his friends during high school and university judo and rugby playing, and also the treatment given by skilled doctors.

However, in 1989 he decided to shift his focus from orthopedic surgery to basic research, due to a self-professed (perhaps tongue-in-cheek) lack of confidence in his own surgical technique. For a long time, he was not a member of the Japanese Orthopaedic Association (JOA) but he returned to the fold in 2009 thanks to active promotion by Professor Kurosaka at Kobe University. As fellow Japanese orthopedic surgeons, we are proud of Professor Yamanaka's Nobel Prize achievement and we congratulate him, as I am sure orthopedic surgeons all over the world would do.

I was fortunate enough to make the acquaintance of Professor Yamanaka 6–7 years ago through a similar field of regenerative medicine. When I invited him to the Hiroshima Orthopaedic Frontier Meeting on November 7, 2007, there were only 50 people in the audience. Several days later, his report on iPSCs from human fibroblasts was published in Cell and it suddenly became difficult to contact him directly. On the occasion of the ninth annual meeting of Japanese Society for Regenerative Medicine in 2010, as Congress President, I invited Professor Yamanaka to hold a special lecture on iPSCs in the largest lecture theater and was surprised that more than 2,000 attended.

The main focus of iPSC treatment is incurable diseases such as Parkinson's disease and amyotrophic lateral sclerosis and on creating novel drugs. To treat an incurable disease – for example a severe cardiac disease with a genetic predisposition – it is essential to know its pathomechanism and the way it progresses from childhood to adulthood. While it is very difficult to obtain cells directly from the heart, it is relatively easy to obtain cardiac cells through the process of converting iPSCs into cardiac cells, which will have the same deficits or problems as the original cardiac cells. Using these cardiac cells, the pathomechanism of the disease can be investigated and the most effective drugs can be discovered.

In the field of orthopedic disease or injury, there are several targets to treat with iPSCs. These cells have a wide spectrum of differentiation from chondrocytes and bone to neurons. At present, it seems difficult to use iPSCs for the treatment of chondral defects, regardless of possible medical trials, since iPSCs are associated with some serious problems such as tumorigenicity. The standard procedures for chondral defects in the knees – where we cannot expect spontaneous healing – are the bone marrow stimulating techniques such as microfracture and drilling, mosaic plasty, autologous chondrocyte implantation developed by Dr. Peterson and Dr. Brittberg et al. and our tissue-engineered cartilage transplantation.

These procedures are selected as appropriate according to the location and size of the chondral lesion and the age of the patient.

Without the assistance of iPSCs, chondral lesions can be successfully treated, although not completely satisfactorily. However, progress with the aid of iPSCs has been more rapid than we had expected. We orthopedic surgeons should continue to search for better treatments for chondral lesions using iPSCs. The research group of Maekawa and Yamanaka successfully reduced the risk of contamination of incompletely reprogrammed cells, which are at risk of tumorigenicity, by introducing Klf4, Oct3/4, Sox2 and Glis1 instead of c-Myc. This technique, though not perfect, can facilitate clinical trials using iPSCs. In 2010, Fukuda's group described a simple procedure to induce mesenchymal progenitors with chondrogenic properties from mouse iPSCs, suggesting that iPSCs may be alternative sources for transplantation of autogenous mesenchymal stem cells to treat cartilage defects.

Although we need to weigh up the risks and benefits, basic research to treat cartilage defects with iPSCs will hopefully lead to a breakthrough in the future. Tsumaki's group recently demonstrated that retroviral induction of 2 reprogramming factors (c-Myc and Klf4) and sox9 which is one of the representative factors for chondrogenesis induced polygonal chon-

DOI 10.3109/17453674.2013.765642

drogenic cells. Some cell lines produced homogeneous hyaline, cartilage-like tissue when subcutaneously injected into nude mice, but other cell lines induced tumor formation. Thus, there have been new technical advances in the area of cartilage formation, but it will take some time for us to solve several issues related to the treatment of cartilage lesions before clinical application will become possible.

The development of an effective therapy for bone defects is also an important goal in the area of orthopedics. Autobone grafts, allobone grafts and artificial bone grafts are used for filling of significant bone defects. However, several problems still remain. The volume of harvested autobone grafts is limited, and the invasion of donor sites is a related issue. Allobone grafts can be infected and they have limited osteoinductive properties. Artificial bone has sufficient strength, but it has no osteoinductive properties. Thus, it is sometimes necessary to use cells such as mesenchymal stem cells and several growth factors. For significant bone defect therapy, we can expect that iPSCs will be able to solve these problems in the future. It has already been reported that osteoblasts derived from iPSCs could show bone formation in bone defects after transplantation of these cells in an animal model. The application of iPSCs derived from patients might help us develop a novel therapeutic strategy for bone defects, fractures, and pseudoarthrosis.

Spinal cord injury may be one of the best targets for iPSCs treatment, since there is no good treatment currently available. In the published reports, iPSCs are transplanted into the injured spinal cord after differentiation to neural stem cell-like cells, which have the potential to differentiate into neurons, oligodendrocytes, and astrocytes. It has been demonstrated that transplanted cells move both proximally and distally, and that they differentiate into 3 types of cells, resulting in functional recovery. The mechanism of functional recovery is thought to be formation of the intraspinal neural circuits by differentiated neurons, remyelination by differentiated oligodendrocytes, and positive effects by humoral factors of transplanted neural stem cells. In addition, it has been reported that growth factors secreted from transplanted neural stem cells have the capacity to promote angiogenesis and neuroprotection.

In the clinical application of iPSCs for the regeneration of spinal cord injury, one of the problems to solve is the timing of cell administration. In 2009, a clinical trial of Geron corporation's ES cell-derived oligodendrocyte progenitor cells to treat acute phase spinal cord injury, was approved, which is intended for patients within 2 weeks of injury. However, according to basic research knowledge about transplantation of neural stem cells, their transplantation in the acute phase when severe inflammation occurs results in a low rate of grafted cell survival. In contrast, it is difficult to regenerate the spinal cord by transplanting neural stem cells during the chronic phase when glial scar has already formed. It is therefore, recommended that administration of cells to the injured spinal cord should be conducted in the sub-acute phase, 1–2 weeks after injury, in rat and mouse models. There is no firm evidence to prove when exactly the sub-acute phase in human spinal cord injury occurs, but it is believed that 2–6 weeks in human spinal cord injury are equivalent to the sub-acute phase, because the sub-acute phase of mice and rats coincides with the period of rapid improvement of motor function. According to international guidelines for the conduct of clinical trials for spinal cord injury, 3 months after spinal injury is set as the time when improvement of function is achieved.

In any case, it takes more than 6 months at present to obtain neural stem cells differentiated by iPSCs induced from somatic cells, and it seems difficult to use autogenous iPSCs for the treatment of spinal cord injury. The most likely procedure will involve allogeneic transplantation of iPSCs after establishing an iPSC bank. In the case of allogeneic transplantation, it is essential to reduce immunological rejection. For this purpose, matching of the human leukocyte antigen between the recipient and the donor is necessary. Although more than 10,000 combinations of HLA types are theoretically possible, each human race has distinctive HLA patterns. For example, in 2008, Nakatsuji et al. estimated that a cell bank size of 50 lines would enable a matched line to be found in almost 90% of the Japanese population.

As mentioned, tumorigenicity is a serious problem, when transplanting iPSC-derived cells. In reports on the transplantation of iPSC-derived neural stem cells in 3 lines and ES cell-derived neural stem cells in 1 line for spinal cord injury of mice, intraspinal tumor formation was observed in 2 lines of iPSC-derived neural stem cells. There was no evidence of tumorigenicity using many iPSC lines, and the probability of tumorigenic transformation was not clear. Nakagawa et al. in 2008 reported that iPSCs could be established using 3 reprogramming factors except c-Myc, which is an oncogene and used as one of Yamanaka's 4 reprogramming factors, to reduce the risk of tumorigenicity. However, in the transplantation of iPSC-derived neural stem cells, intraspinal tumor formation was observed, even though the iPSC-derived neural stem cells were established by 3 of Yamanaka's 4 reprogramming factors except c-Myc. Therefore, before start of a clinical trial, tumorigenicity analysis is necessary in each cell line prior to transplantation. Furthermore, we need to find a way of reducing the risk of contamination by undifferentiated cells to almost zero. Unlike embryonic stem cells obtained from a later destroyed fertilized egg, iPSCs originating from our fibroblasts present no ethical problem.

In the clinical setting of the transplantation of iPSC-derived cells, there are problems not only regarding safety but also regarding the need for many homogenous iPSCs to regenerate organs. The development of a large scale culture system to obtain many homogenous iPSCs should be established for clinical application. When these problems have been solved, treatment in the orthopedic field will change dramatically. In the future, a large number of chondrocytes derived from iPSCs will be obtained from patients and used for significant

cartilage defects. This could be a novel effective therapy for osteoarthritis, which afflicts millions of patients worldwide. In addition, iPSCs generated from patients could be differentiated into specific cells expressing specific phenotypes as a disease model to use efficient drug screening for incurable diseases.

In 2000, the WHO endorsed the ”Bone and Joint Decade”, as had been proposed by Professor Lidgren of Lund University, Sweden. This “Decade” was observed in 97 countries including Japan to promote musculoskeletal health. Musculoskeletal disorders cause a reduction in the working population and an increase in the number of elderly people with a walking disability and who require nursing care. Japan in particular has become an ageing society even more than other countries. In 2011, the population of elderly people over 65 years of age reached almost 30,000,000, accounting for one quarter of the total population. In an ageing society such as that in Japan, many elderly people suffer from musculoskeletal disorders such as osteoarthritis, osteoporosis related fractures and spondylosis. Thus, the JOA proposed and promoted use of the term “Locomotive Syndrome”. The locomotive organs consist of 3 components; bone, joints, and muscles and nerves. A disorder involving these elements causes walking disability, eventually requiring nursing care. Thus, there has been a campaign to check the signs and symptoms of locomotive syndrome and to recommend locomotion training. Furthermore, the role of orthopedic surgery in the regeneration of locomotive organs is increasingly important in a super-aged society. The discovery of iPSCs will enable regeneration of locomotive organs and will lead to a reduction in the proportion of elderly people with a walking disability, and who need nursing care. This in turn will contribute not only to the quality of life of individuals but also to the welfare of the nation.

iPSCs will bring innovation to regenerative medicine and drug development in the near future. Also, the use of iPSCs will drastically change orthopedic strategy. They provide hope in the conquest of incurable diseases and I sincerely hope that Dr Yamanaka’s achievement will prove to be a big turning point in medical history.

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