Research that Supports Our Fundamental Understanding of iPS Cells

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Autumn 2014 was an eventful autumn with a huge impact on induced pluripotent stem (iPS) cell research. First, as an undertaking in clinical research, the world's first transplant involving iPS cell-derived cells was performed. In another development, drug discovery research with iPS cells led to the first-ever report demonstrating the potential effectiveness of using existing drugs to treat different diseases. We are fast approaching the day when iPS cell research will at last be of genuine value to many patients. It is worth noting, that the Grants-in-Aid for Scientific Research (Kakenhi) have been instrumental in paving the way for progress in this field.

My first experience with Kakenhi was a Grant-in-aid for the Encouragement of Scientists (A) that I received after I had returned to Japan from overseas study in the US and begun pursuing research at Osaka City University. I utilized that funding to investigate the functions of NAT1, a gene that had been identified as a candidate tumor suppressor. At that time, I had been using embryonic stem (ES) cells from mice for my research but had been advised that I should focus my research more on human cells than cells from mice. Basic research such as this tends not to produce immediate or tangible results and I recall struggling to win acceptance from my colleagues for the direction I wanted to take. Despite this setting, continued grant funding enabled me to move my research forward without interruption.

In 2004, I pursued an investigation into the characteristics of ES cells with grant funding for a project under the Grants-in-Aid for Scientific Research (B). ES cells are pluripotent in that they can differentiate into all types of cell in the body; furthermore, they are able to proliferate almost indefinitely. In 1998, it was reported that the establishment of human ES cell lines was successful. This sparked hopes that ES cell therapies would find use in regenerative medicine. However, because the generation of ES cells relied on the use of human fertilized eggs, in certain nations research in this field was tightly regulated.

My research group had the idea that it might be possible to derive cells with characteristics identical to ES cells, but from somatic cells rather than fertilized eggs. Utilizing a database of gene expression data, we began searching for genes specific to ES cells. Next, we applied a

method to experimentally confirm our findings, and from a candidate set of approx. 30,000 genes, eventually identified over 20 genes with signature expression in ES cells. Refining our focus, we then discovered four genes that were necessary to reprogram somatic cells into cells with the characteristics of ES cells. They were Oct3/4, Sox2, Klf4, and c-Myc. iPS cells were generated through the introduction of these four "factors" into skin cells.

In 2006, our research group reported success in generating iPS cells from the dermal cells of mice. This triggered a global race to produce human iPS cells. In 2007, we reported success in generating iPS cells from human dermal cells utilizing a similar protocol but on that same date, a research group in the US also reported that it had generated human iPS cells using a combination of different genes. Given that much of the accumulated knowledge from ES cell research applied directly to iPS cells as well, iPS cell research began expanding dramatically on a global scale.

In 2007, we gained a powerful source of financial support for its research on iPS cells with a grant for work in the category of Grants-in-Aid for Specially Promoted Research. Although the early lines of generated iPS cells were prone to mutations with a higher risk of tumorigenesis, techniques were later developed to produce iPS cells using safer methods that lowered the risk of tumor formation. This and other achievements enabled us to pursue research that would form a solid foundation of knowledge for the clinical application of iPS cell therapies.

Producing iPS cells required the insertion of factors into target cells. Initially, we had utilized retroviruses for that purpose. However, on rare occasions, inserted genes could cause damage to the original cell genome. Moreover, one of the factors inserted—c-Myc—was already known as an oncogene. Consequently, with the early methods, the transplantation of iPS cell-derived cells into mice sometimes could lead to cancer.

Now, we use episomal plasmid vectors that do not cause genetic damage to the target cell's genome. Factors inserted with this approach neither harm the original cell genome nor do they even remain inside the cell. We also have generated iPS cells by replacing the c-Myc oncogene with L-Myc, a similar factor that is thought to pose a lower risk of inducing cancer. Thanks to these changes, we have succeeded in adequately ensuring the efficiency of the iPS cell generation process and obtaining iPS cells marked by a lower risk of tumorigenesis.

As one outcome of these developments, in 2014—seven years following the release of our study on the successful induction of pluripotent stem cells from adult human cells—the world's first

transplant using iPS cell technology in a human patient was performed by a group led by a researcher at RIKEN. Although this transplantation is clinical research that is still in the stage of answering questions about safety, the abundant levels of Kakenhi that we received for basic research immediately following our success in generating iPS cells was, I think, a key factor that enabled us to move forward with the study of clinical applications.

I suppose opinions vary about Kakenhi but as I see it, the recent creation of the Grant-in-Aid Fund for certain categories of research has, among other steps, significantly improved the convenience of grant funding. I want to see Kakenhi continue to be administered flexibly according to future needs and accordingly serve as a foundation of support for scientific research in Japan. I also feel it will be necessary to establish mechanisms or frameworks of some kind that can allocate adequate levels of research funding to undertakings in basic research that do not always readily demonstrate clear-cut benefits.

The Center for iPS Cell Research and Application at Kyoto University invites donations from the general public and is working to diversify its sources of research funding. Japan does not yet appear to have a well-established culture of donations and has not always shown much success in raising donations compared to nations in the West. Donations from the general public are an effective source of funding for research that is perceived as having clear objectives and understandable outcomes. By contrast, it is more difficult to acquire funding for efforts in basic research where the benefits are not immediately apparent or forthcoming.

It is my hope that Kakenhi will be allocated more enthusiastically to projects in basic research that can be expected to support Japanese society in the years ahead.