Shinya Yamanaka, M.D., Ph.D. - 2012 Nobel Prize Laureate: How his dream of a research career provides vision for the next generation of young scientists

2012 Nobel Ödül Laureat`i Dr. Shinya Yamanaka`nın araştırma kariyeri düşleri gelecek nesil genç bilim adamlarına nasıl bir vizyon aktarıyor

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The career path of Dr. Shinya Yamanaka started with a dream to be a scientist and led to him receiving the 2012 Nobel Prize in Physiology or Medicine. Dr. Yamanaka discovered how to convert skin fibroblasts into induced pluripotent stem (iPS) cells, which are embryonic-like stem cells that could become any of the more than 120 different cell types in the body. His ground-breaking finding, made through hard work, dedication, and a willingness to follow the research wherever it led him, has changed medicine forever. There is much to be gained by young scientists around the world, including Turkey, from Dr. Yamanaka's career path and lessons learned, which can be summarized in just a few words: develop a vision and work hard to achieve it.

Postdoctoral Fellowship at the Gladstone Institutes in San Francisco

The year was 1993, and no one was returning Dr. Shinya Yamanaka's phone calls. The physician and recent Ph.D. graduate of Osaka City University had given up a promising career as an orthopedic surgeon to pursue his dream as a research scientist. But now he was not receiving any responses to his application to become a postdoctoral research fellow in the United States.

Until, that is, Dr. Yamanaka's application came across the desk of Thomas Innerarity, Ph.D., a senior investigator focused on cardiovascular research at the Gladstone Institutes in San Francisco. "I applied to many, many research institutes in the United States, but I received no responses," recalled Dr. Yamanaka. "The one exception was Tom, who invited me to come to Gladstone and be a part of his research laboratory."

It was a wise decision. Founded in 1979, Gladstone is an independent, nonprofit research institute that focused primarily on research into cardiovascular and viral diseases at the time (later adding a third focus on neurological diseases in the mid-1990s) (www.gladstoneinstitutes.org). The work Dr. Yamanaka undertook as a Gladstone postdoctoral fellow – and the Gladstone notion that researchers must follow science wherever it leads – would form the basis for his subsequent discovery of how to reprogram adult skin cells into iPS cells. Now back at Gladstone as a senior investigator, this former Gladstone postdoctoral fellow has won the 2012 Nobel Prize in Physiology or Medicine.

When Dr. Yamanaka first arrived at Gladstone for his postdoctoral training more than 20 years ago, stem cells were far from his mind. Rather, Dr. Yamanaka focused his research on finding new ways to decrease the levels of low-density lipoproteins (LDL), a major risk factor for coronary artery heart disease. It was widely known among scientists that a protein called apolipoprotein (apo) B was the principal component of LDL. Gladstone scientists were investigating two forms of the apoB protein that existed in the body. The longer form (apoB100), normally found in the liver, is the structural protein of LDL and is recognized by LDL receptors that take up cholesterol. Another shorter form, apoB48, is the structural protein of chylomicrons and is synthesized in the intestine. Importantly, it does not bind to LDL receptors, making it less likely to contribute to cardiovascular disease. Dr. Yamanaka sought to understand how both forms were synthesized. If he could understand that, he reasoned, he could then find ways to change the longer form so that it would resemble the shorter and harmless form and reduce the risk of heart disease.

In early experiments, Dr. Yamanaka studied an enzyme called APOBEC-1, which facilitates synthesis of the shorter apoB48 form. In the liver, APOBEC-1 is normally inactive, and the

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Figure 1. Dr. and Mrs. Robert Mahley and Dr. and Mrs. Shinya Yamanaka
(Photo taken in Stockholm, Sweden, on the occasion of the awarding of the 2012 Nobel Prize in Physiology or Medicine.)

longer apoB is synthesized in this organ. Working under Dr. Innerarity's guidance, Dr. Yamanaka and the other scientists in the laboratory thought that by activating the APOBEC-1 enzyme in the liver, they could mimic the intestinal process to create a shortened form of the apoB protein that would lower LDL levels and thus decrease the incidence of heart disease.

A failed scientific experiment?

The entire laboratory staff began planning and carrying out the experiments needed to test this hypothesis. Twenty years ago, experiments were very labor intensive because many of the reagents had to be prepared in the laboratory – things that today can easily be ordered from chemical supply catalogues. The research team would line up along the entire laboratory bench like an assembly line, with each person, including Dr. Yamanaka, taking on a different step in the experiment. The team's experiments on mice did show a reduction in LDL but, unpredictably, the experiments also had an unintended side effect – the mice developed hepatocellular carcinoma (1), which was a big disappointment to all. Obviously, this approach would not be useful in treating heart disease. Instead of getting discouraged, Dr. Yamanaka, who had worked the hardest on this project, got curious. He wanted to understand what went wrong.

The Gladstone philosophy has always been to give scientists-in-training, like Dr. Shinya Yamanaka, the freedom to follow wherever their curiosity and the science lead, even if that means switching research focus, primary methods, or disease expertise. So for Dr. Yamanaka, this latest turn of events did not constitute a failed experiment; but instead led to the beginning of a new project. This curiosity and willingness to pursue a research project, even in the face of what others would consider a failure, have played a big part in making him the scientist and the person he is today.

Lessons learned by a young scientist

One of my roles as director of the Gladstone Institute of Cardiovascular Disease and president of the Gladstone Institutes was to encourage young scientists like Dr. Yamanaka. One year at our scientific retreat I told them about the secret of having a successful scientific career: "VW." It had nothing to do with the fact that I always drive a Volkswagen (VW) vehicle; rather, VW stands for vision (V) and hard work (W). Dr. Yamanaka has often said that one of the important lessons he learned at the Gladstone was the notion of VW, which guided him even through difficult times. By moving on from what some considered a failed project with a new vision and hard work, he truly embodied this notion. Today, Dr. Yamanaka frequently stresses to young scientists that being a successful researcher and human being requires a clear vision and a willingness to work hard to fulfill it.

A new path opens new doors

Dr. Yamanaka wondered if switching on APOBEC-1 in the liver was responsible for the tumor growth in the liver. Further research refined his theory: he found that switching on APOBEC-1 in the liver altered NAT1, a protein that, when modified, causes tumors to develop. Dr. Yamanaka had therefore found the cause of the tumor growth: expression of a dysfunctional NAT1 (2). Now he wanted to find the solution.

Dr. Yamanaka wanted to study mice that lacked NAT1 to see whether they would still develop cancer. But to do so, he needed to develop genetically modified mice – a process that required embryonic stem (ES) cells. ES cells are called pluripotent, meaning that they have the ability to develop into any type of cell, such as skin, muscle, heart, or blood cells. To obtain the ES cells he needed for his study, Dr. Yamanaka collaborated with Robert Farese, M.D. a Gladstone investigator whose laboratory was the only one with the tools to develop ES cell cultures. He learned every aspect of this relatively new technique, from generating and culturing the human ES cells to inserting the specific constructs lacking NAT1 into mouse eggs. However, he was not able to reach the last stage of creating the genetically modified mice because the NAT1-lacking ES cells that he developed never matured; they just multiplied over and over again. Thus, by accident, he discovered that NAT1 was key to helping stem cells transform into individual cell types. After he made this important observation, he turned his central research focus to discovering the genetic factors involved in this process.

Back to Japan to forge his vision for the future

His new direction eventually took him back to Japan where, armed with the expertise gained at Gladstone, he undertook what seemed to be an impossible undertaking: to find out which genetic factors from among hundreds of possibilities instruct ES cells to become other types of cells. He sought to understand the molecular mechanisms that underlie the pluripotency and rapid proliferation of ES cells and to identify the factors that induce reprogramming. This laborious task involved sorting through many different factors that influence cell development and using them to reprogram somatic cells into ES-like cells. Dr.

Yamanaka selected the most likely ones and began examining them in various combinations. With what was widely seen as stunning speed, he first announced the four genetic factors behind his iPS breakthrough in mice in 2006 (3).

Dr. Yamanaka reported that adult mouse fibroblasts could be induced to form pluripotent cells by expressing just four genes (Oct4, Sox2, Klf4, and c-Myc). The next question Dr. Yamanaka addressed was whether his method would work with human cells. In the past, attempts to translate other findings (e.g., nuclear transfer) from mouse to human cells had been extremely difficult to carry out. However, in November 2007, Dr. Yamanaka electrified the world with the news that iPS cells could be made from human skin cells with the same factors used in mice (4). The ease with which the iPS cell discovery was translated to human cells emphasizes the fundamental importance of his original work in mice. Dr. Yamanaka subsequently found that one of the factors, c-Myc, was not needed, and that reprogramming could occur in the absence of any genomic integration events, and this was confirmed by others. Also in 2007, he showed that iPS cells can produce fully reproductive mice – definitive proof that iPS cells are pluripotent (5).

Dr. Yamanaka's original report was met with surprise and disbelief. The vast majority of scientists believed that nuclear reprogramming would require the use of early ES cells, via nuclear transfer or cell fusion. Major efforts on three continents to make nuclear transfer work in human cells had limited success. Most biochemical efforts focused on identifying complex combinations of "reprogramming factors" in the cytoplasm of fertilized eggs. Thus, Dr. Yamanaka's announcement that he could induce iPS cells from skin cells with a simple cocktail of four genes seemed too good to be true. His method (starting with skin fibroblasts) was radically different from those of other scientists (nuclear transfer, cell fusion), and the combination of genes seemed much too simple. However, in short order, Dr. Yamanaka provided reagents to other laboratories, and his results were rapidly confirmed.

Dr. Yamanaka's discovery altered the way scientists think about the processes that control activation and repression of genes: only three factors are sufficient to epigenetically "erase and re-mark" the entire genome. The simplicity of the final method emphasizes the beauty and complexity of human beings.

Dr. Yamanaka returns to his scientific roots: Gladstone

Dr. Yamanaka was recruited to Gladstone in 2007, this time as a senior investigator and a professor of anatomy at the University of California, San Francisco, with which Gladstone is affiliated, while still maintaining a laboratory in Kyoto.

The development of iPS cell technology by Dr. Yamanaka represents an entirely new platform for studying the fundamental mechanisms of human diseases and for developing therapies to overcome them. Rather than using models made in yeast, flies, or mice, which do not always recapitulate the features of human disease, this technology allows human stem cells to be created directly from patient tissues. As a result, these cells contain a complete set of the genes that resulted in a specific disease, potentially offering a far-superior human model for studying disease and testing new drugs and treatments.

While the application of iPS cells to regenerative therapies will require refinement, iPS technology is fundamentally altering the way scientists work. Dr. Yamanaka's discovery of iPS cells is transforming multiple disciplines, from basic biology to regenerative medicine, and its simplicity has encouraged other researchers into this field, thereby accelerating progress in this field of research. Today, laboratories across the world are racing to generate iPS cells from humans with all types of disease, investigating cellular pathologic mechanisms in cells containing the disease-causing genetic background, and differentiating disease-specific iPS cells to screen for novel therapeutic agents. Finally, iPS technology provides the exciting possibility of harnessing the benefits of stem cells by producing patient-specific pluripotent cells for cell-based therapies that can avoid immunorejection and other biochemical issues.

Today, Dr. Yamanaka splits his time between San Francisco and Japan, where he is director of the Center for iPS Cell Research and Application at Kyoto University. After years of hard work and international recognition, his principal desire–to find workable solutions to pressing medical problems has never wavered.

On winning the 2012 Nobel Prize in Physiology or Medicine, Dr. Yamanaka stated: "The best part about this prize is that it will bring attention to, and will likely spur, the important stem cell work that scientists around the world are conducting. This iPS technology is for patients, and the more scientists who build on it, the faster we can help those who live with chronic or life-threatening diseases."

Advancement of science by nurturing the next generations

Mentoring, encouraging, and instructing young scientists is like planting trees, which give shade that extends beyond what we might imagine. There is no greater reward for a professor or teacher than participating in planting careers of young scientists in fertile soil. The benefit of the shade has the potential to relieve the suffering of our patients from multiple diseases.

Congratulations, Shinya Yamanaka, for making a difference!

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