Radical treatment for leukemia under way

January 17 2011, By Fumihiko Ishikawa

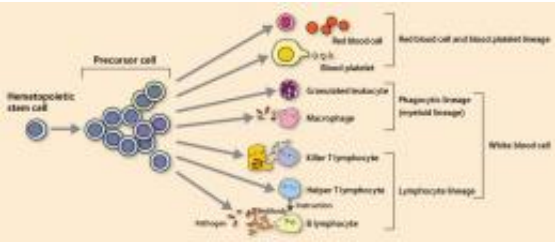

Figure 1: Major blood cells that differentiate from hematopoietic stem cells. Hematopoietic stem cells differentiate into various types of blood cells. Acute myelocytic leukemia develops when phagocytic-lineage cells become cancerous.

Humanized mouse models help clarify the origins of leukemia and the cellular processes that lead to its recurrence, providing hope for a cure for this intractable blood disease.

When viruses or bacteria enter the body, they are eliminated by white blood cells. Yet white blood cells, which are so instrumental to our immune function, can become cancerous and proliferate abnormally and uncontrollably, resulting in a loss of the ability to produce normal blood cells. This disease is called leukemia, and it can occur in people of almost any age, from infants to the elderly. Several types of this intractable disease exist, including acute myelocytic leukemia, which has a higher incidence in adults. About three in every 100,000 people are thought to develop the disease. A small number of leukemia patients can now be cured completely through anticancer drug treatment or bone-marrow transplants, but acute myelocytic leukemia has a particularly

high relapse rate, leading to death in many cases. In 2007, Fumihiko Ishikawa and the members of his Research Unit for Human Disease Model at the RIKEN Research Center for Allergy and Immunology (RCAI) discovered that the major cause of this high relapse rate lies in leukemia stem cells, which are resistant to anticancer drugs. In 2010, they presented new research results on two approaches to killing leukemia stem cells.

After graduating from the Faculty of Medicine at Kyushu University in 1997, Ishikawa began to work as a clinician in charge of leukemia treatment in the First Department of Internal Medicine at Kyushu University's Faculty of Medicine. "I used to share wonderful experiences with patients and their families when our treatment with anticancer drugs greatly improved patients' symptoms. They were indeed amazing experiences for a doctor, but I thought that we should not be satisfied with this."

Some patients experience a recurrence of leukemia even when their symptoms have been greatly improved. Acute myelocytic leukemia has both high recurrence and mortality rates. "Patients and their families are leading their lives in fear of a recurrence of the disease. This led me to think that basic research is indispensable to prevent the disease from recurring, and that finding a radical treatment for the disease is of utmost importance."

In 1998, Ishikawa moved to the Medical University of South Carolina in the USA, where he started his studies on humanized mice in order to analyze the human immune system using mouse models. "Mice are used in many studies to understand biology in vivo and find effective ways to overcome diseases. For example, researchers create model mice and develop particular diseases in them in place of human patients. However, the findings from mouse studies are not always applicable to medical care or drug discovery. This is why I wanted to attempt to recreate the

human immune system in a mouse."

The research theme was quite challenging. In 1988, a research group led by Stanford University in the USA published a pioneering work on reconstitution of human immunity in mice. As the engraftment levels of human cells were not that high, investigators attempted to improve the in vivo assay in varous ways. Ishikawa's attempt to create an immunologically humanized mouse took a different approach. "When human cells are transplanted into a mouse, the cells are rejected by the mouse's immune system. To avoid rejection, we need to create an immune-deficient mouse, or an immune-suppressed mouse. Then we can transplant hematopoietic stem cells from humans into the mouse to create various blood cells, including human-derived white blood cells."

There are many types of blood cells, including white blood cells, red blood cells and blood platelets. White blood cells are responsible for the immune response and can be grouped into phagocytic cells, which ingest and consume foreign bodies, and lymphocyte cells, which attack foreign bodies (Fig. 1). The various types of blood cells all are produced by hematopoietic stem cells. "The exact location of hematopoietic stem cells was not clearly known, which made it difficult to extract them from surrounding tissue. I searched for molecules that could serve as markers for hematopoietic stem cells, and in doing so moved our own research forward."

In 2002, Ishikawa had a chance to meet Leonard Shultz from the Jackson Laboratory in the USA. "Doctor Shultz is an authority on immune-deficient mouse development. We got along well because of our shared desire to overcome leukemia and a belief that the key to achieving this goal is to search for the pathogenesis of the disease by studying immunologically humanized mice. We have been collaborating in our research ever since." Leonard Shultz has been supporting the research activities of the Ishikawa's laboratory through the creation of

various new strains of immune-compromised mice and constructive discussion.

Ishikawa returned to Japan in 2002 and again started to work as a physician scientist in the First Department of Internal Medicine at Kyushu University's Faculty of Medicine. He also teamed up with young medical doctors and graduate students in the First Department and pursued his studies on immunologically humanized mice. "We worked not only on developing a technique for extracting hematopoietic stem cells from humans selectively, but also on a technique for transplanting those stem cells into newborn immune-deficient mice by injecting the cells into the mouse's blood vessels. We also had discussions with Dr Shultz to decide what kind of immune-deficiency mice should be developed in order to devise treatments for human diseases including recurrent leukemia. In 2005, we successfully created a prototype of an immunologically humanized mouse." In their model immunologically humanized mouse, human-derived white blood cells account for 80–90% of the white blood cells.

Pointing the finger at leukemia stem cells

It was Masaru Taniguchi, director of the RIKEN RCAI, who drew special attention to the center's studies on immunologically humanized mice. In 2006, Ishikawa started his own research unit at the RCAI and began to work on creating leukemia humanized mice, that is, mice with human acute myelocytic leukemia.

Leukemia has been thought to be caused by the continuous proliferation of leukemia cancer cells. In recent years, however, it has become known that the large numbers of leukemia cells found in cases of acute myelocytic leukemia are produced by just a small number of leukemia stem cells.

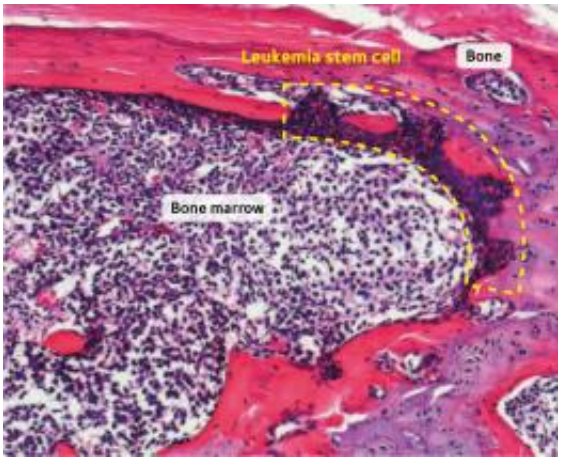

Figure 2: Leukemia stem cells that survive treatment with anticancer drugs. Treatment of leukemia humanized mice with anticancer drugs can kill many leukemia cells, but 70-80% of leukemia stem cells survive, concentrating in the niche between the bone and bone marrow.

“We extracted leukemia stem cells from the bone-marrow fluid of an acute myelocytic leukemia patient and transplanted them into newborn immune-deficient mice. The mice subsequently developed symptoms similar to human acute myelocytic leukemia.” Ishikawa and his team successfully created leukemia humanized mice in 2007 by applying the same technique as used for generating immunologically humanized mice.

“The mice developed leukemia after receiving about 1,000 leukemia stem cells, but did not develop the disease when injected with just the leukemia cells themselves, even when we transplanted over a million such cells. This result clearly showed that the stem cells are the pathogenesis of leukemia. Various studies on mice now suggest that leukemia stem cells result from some abnormality occurring in the genes of hematopoietic stem cells or precursor cells that eventually differentiate into various blood cells.”

Ishikawa and his team have also succeeded in clarifying the reason for the recurrence of leukemia. “Most leukemia cells other than stem cells are eliminated when we administer anticancer drugs to leukemia humanized mice during studies that recreate leukemia treatment for human patients. However, we found that 70–80% of leukemia stem cells survive treatment, and these continue to go on producing large numbers of new leukemia cells. This is how we discovered that the recurrence of leukemia is caused by the leukemia stem cells. The anticancer drugs used currently are ineffective against leukemia stem cells.” The team also found that the leukemia stem cells that survived the treatment with anticancer drugs gather in the boundary region (niche) between the bone and the bone marrow (Fig. 2).

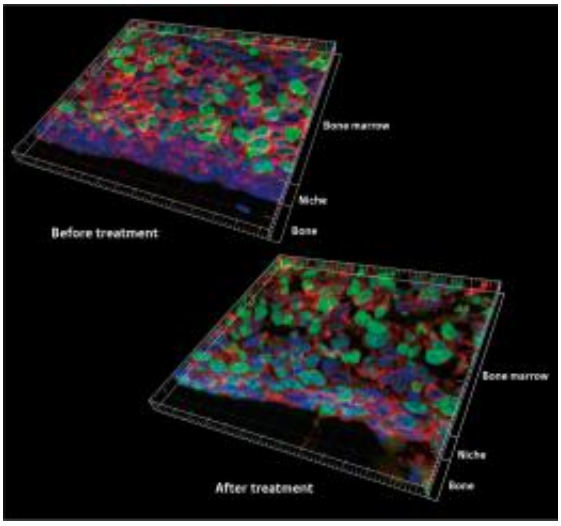

Figure 3: Composition of bone marrow in leukemia humanized mice. Before cytokine treatment (left), cells with an active cell cycle (green) are absent from the niche, where leukemia stem cells concentrate. After cytokine treatment, the cell cycle is restarted in niche cells (blue, cells within the bone marrow; red, white blood cells).

It is controversial whether cancer stem cells could be the pathogenesis for cancers other than leukemia. “It is well-accepted, however, that leukemia stem cells are the pathogenesis of acute myelocytic leukemia in adult patients because all of the different types of blood cells are derived from hematopoietic stem cells. Our research results strongly support this fact.”

Killing leukemia stem cells

Investigations in Ishikawa’s research unit then turned to examining the reason why anticancer drugs are ineffective against leukemia stem cells. Cancer cells, unlike normal cells, generally undergo repeated cell division and proliferation. In 2010, however, Ishikawa and his team found that leukemia stem cells in the bone marrow of leukemia humanized mice did not undergo this cell cycle of cell division and proliferation. “Researchers have developed anticancer drugs that target the cancer cells that divide and proliferate at high rates. These drugs can be considered to be ineffective against leukemia stem cells, which have a paused cell cycle.”

Ishikawa tried administering a protein called cytokine to stimulate cell division and proliferation in leukemia humanized mice. Yoriko Saito, a senior researcher in the Ishikawa Unit, demonstrated through confocal imaging that treatment restarted the cell cycle of leukemia stem cells (Fig. 3) and that subsequent treatment with anticancer drugs killed many of the leukemia stem cells. “It has been known empirically at clinical sites that a combination of cytokine and anticancer drugs is effective.” Although promising, however, this approach does not perfectly kill off all leukemia stem cells. “The effect depends on the patient,” says Ishikawa. “Since there are various kinds of cytokines, one approach could be to find the best combination of cytokines for individual patients. We think that separating leukemia stem cells from their niche site between bone and bone marrow could also be effective because it

may be that certain molecules in the niche act on the leukemia stem cells to stop their cell cycle. Separating leukemia stem cells from the niche may restart the cell cycle."

In cooperation with Osamu Ohara, group director of the RCAI Laboratory for Immunogenomics, Ishikawa and his team have also been targeting leukemia stem cells directly. "We compared the genes of leukemia stem cells with those of normal hematopoietic stem cells and determined which genes are activated only in the leukemia stem cells. We also used various approaches to narrow down the genes to 25 molecules that express themselves only in leukemia stem cells." These molecules include those that express themselves on the surface of leukemia stem cells and enzymes essential for the leukemia stem cells' functioning and survival. Effective approaches can be devised based on this discovery, such as developing drugs that can combine with the molecules on the surface of the leukemia stem cells, or those that can inhibit the functions of the enzymes. "We will have found a complete cure for leukemia if such drugs can kill the leukemia stem cells."

In April 2010, RIKEN started its Program for Drug Discovery and Medical Technology Platforms aiming to support studies on drug discovery. "Our studies have also been selected as research themes that are to be supported. For example, we can take advantage of immunologically humanized mice or leukemia humanized mice to recreate various clinical conditions on an individual patient basis and try out the effects of candidate compounds under development or various treatments on these mice. We can also take advantage of these humanized mice to determine the best drugs or treatments for individual patients, which could lead to the development of personalized medicine. This program will surely advance studies towards a radical treatment for leukemia."

Ishikawa and his team are also improving their leukemia humanized

mice. “In conventional leukemia humanized mice, the molecules in the niche where leukemia stem cells are concentrated are mouse-derived. Thus, we are attempting to replace the mouse-derived molecules with human-derived molecules to develop a new generation of leukemia humanized mice.”

Finding a radical treatment for leukemia through research on leukemia stem cells

In parallel to his studies at RIKEN, Ishikawa is communicating with clinicians. “We cannot make use of our research results or advance our own research without listening to the opinions of clinicians. This is why we are conducting our research in cooperation with Shuichi Taniguchi’s hematology division at Toranomon Hospital, which boasts outstanding achievements in the treatment of leukemia.”

Ishikawa and his team are moving ahead with their research with a strong will to achieve a cure for leukemia as soon as possible. “However, acute myeloid leukemia in adults is one of the most intractable malignancies. It has yet to be beaten despite a long history of medical research. We need to focus not only on drug discovery and clinical applications, but also on the essence of leukemia stem cells that cause the disease. We will then be able to find a new rational approach toward an effective treatment. We need effective approaches from different perspectives in order to overcome the difficult challenge that leukemia presents.”

Provided by RIKEN