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KOL Interview

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HEALIOS K.K. KOL Interview

Live cell analysis that robustly captures the behavior of living cells - Aiming to develop new cancer therapies using NK cells

Dr. Yu-suke Torisawa, HEALIOS K.K.

HEALIOS K.K., a company that is developing products for regenerative medicine using iPS cell technology, is conducting research and development on immunotherapy for a wide range of cancer diseases using their iPS cell-derived NK cells, whose functions have been enhanced through gene transfer and editing. Doctor Yu-suke Torisawa, an expert in the Exploratory Research Group at the Research Department of the company, had encountered difficulties in capturing movement functions such as migration displayed by NK cells under a microscope, while conducting research on cancer therapy with NK cells. It was during that time that he discovered the Incucyte® SX5 Live-Cell Analysis System from Sartorius Japan K.K., which solved various problems and accelerated his research. We interviewed Dr. Torisawa about the usage and benefits of this system.

—— Please tell us about your research, Dr. Torisawa.

I was involved in constructing systems for evaluating migration and other functions of T cells at my previous job, and am currently involved in the functional evaluation and production of NK cells at the company. Our goal is to develop new cell therapies that can be used to treat many types of cancer, including solid tumors, by producing functionally enhanced NK cells from iPS cells. Currently, cell therapies using genetically modified T cells, including CAR-T cell therapy, are performed in clinical practice. However, since T cells recognize self and nonself, these therapies commonly need to be tailored using the patient's own cells. In addition, there are concerns regarding serious adverse reactions. With NK cells, such adverse reactions are almost completely absent, even when using cells from others, and the development of off-the-shelf products is possible. Clinical studies on NK cell-based therapies are also being started.

—— What kind of NK cells are you designing?

We are developing NK cells by producing iPS cells that are transfected with genes which enhance cell functions, such as the ability to attack cancer cells and to survive for a long period of time within the human body, and then inducing their differentiation into NK cells. The goal is to develop NK cells that can target various types of solid cancers. We have already developed NK cells that can recognize and attack cancer, and are evaluating these cells using the Incucyte® SX5 Live-Cell Analysis System.

The Incucyte® SX5 Live-Cell Analysis System, which can capture the behavior of NK cells with high throughput and cost-effectiveness

—— What aspects of the system are suitable for NK cell evaluation?

First of all, it is very well suited for evaluating cell migratory function. In my previous job, I evaluated the migratory function of CAR-T cells moving towards cancer under a microscope while culturing them. After joining HEALIOS in 2020, I attempted to evaluate NK cells under a microscope in the same way. However, I encountered difficulties in observing the cells, because they shift or change position due to movement of the observation stage. Cancer cells, for instance, are adherent and are not affected as much by stage movement, but it is difficult to follow the movement of floating cells such as immune cells and blood cells under a microscope.

In the case of the Incucyte® system, it is the optics, rather than the stage, that moves, so we can observe and evaluate floating cells, which remain undisturbed. In addition, culture under a microscope is harsh for cells and does not allow for a long period of observation, but this problem can also be overcome by using this system, which enables measurement in an incubator. Its ability to support the simultaneous observation of six microwell plates is also very much appreciated. With a microscope, only one image can be viewed at a time. Even with time-lapse microscopy, only one plate can be handled automatically. Considering you look at several plates per day, this is definitely inefficient. In addition, all settings beginning with focusing are performed manually, as you know, and there is a natural limit to the amount of data that can be obtained. Moreover, when it comes to evaluating migratory function, both video creation and quantitative evaluation are carried out manually, usually by a single person. With Incucyte®, however, all operations including focusing and filming are performed automatically, and experiments can be carried out with all six microplates simultaneously. I wonder how much time, effort, and manpower would be needed if we attempted to do the same with a microscope. As Incucyte® can do this work both day and night, as well as on holidays, I think it is the most appropriate device for evaluating living cells.

—— Manual work when performing microscopic evaluation is also highly person-specific.

For example, if you specialize in video and want to take clear images to show, use of a microscope with high resolution may be better due to its ability to set the focus exactly as desired. However, use of a microscope is less reproducible for quantitative evaluation. Academic research may require better data, or clearer data in some cases, but companies that deal with pharmaceuticals should emphasize reproducibility, where the same value is obtained without inter-individual variation. Therefore, evaluation with Incucyte® is considered to be fairer.

—— Even in academic research, reproducibility needs to be emphasized if the research is to become a seed for drugs.

I agree. I think it is still important to be able to evaluate quantitatively.

Incucyte® Live-Cell Analysis System

—— What was the trigger for introducing Incucyte®?

I heard that Incucyte® had been listed as a candidate for the next new device around the year before I joined HEALIOS. It was a hot topic within the company; so, I became aware of it. Furthermore, a group in the U.S. that was producing NK cells from iPS cells was using Incucyte® for cell evaluation, and published an article on their work. When I read it, I became convinced that this system would be useful for us, and I introduced it in 2021.

A "trial," or prospective run for the automatic acquisition of images can lead to a breakthrough

—— What benefits have you gained by being able to leave more work to the Incucyte® Live-Cell Analysis System?

Being able to automatically acquire image data over a long period of time and analyze the data in parallel is extremely efficient, and is of great benefit to the company in terms of cost performance. As a researcher, I feel that the ability to increase the number of experiments is a major advantage. Previously, we had to perform a single experiment under a single microscope, and then evaluate the results to some extent prior to moving on to the next. So, our only option was to proceed sequentially. In contrast, with Incucyte®, multiple experiments can be run in parallel, and analysis results can be obtained while the experiments are underway. Thus, we are able to move

on to the next experiment quickly, and perform multiple experiments seamlessly. Personally, I feel that automatic image acquisition is a great advantage. Although it may not be quite common usage, I often use it when I want to "just give it a try." For instance, if I have a few samples, I can place them into Incucyte® and take some images, perhaps a few per day, for the purpose of a trial evaluation, without quantitation. This is easy to do, and we can also run such a trial in the unused space on a running plate. Later on, when we examine the images in the program, we may discover something that leads to the next experiment or gives us a new idea. I feel this is a big advantage, because it was very difficult to "just try things out" using a microscope.

The Incucyte® Live-Cell Analysis System

—— It gives you more chances to try. Could this lead to a breakthrough?

Possibly. We can try a variety of uses, because the system is not for a limited set of applications. Moreover, I think it is also beneficial to have simple results that are visually understandable.

—— Have you obtained any specific ideas from such "trials"?

Evaluating the migratory function is exactly what this case is about: it became possible through such a trial. Our NK cells have the ability to secrete substances that recruit other immune cells. However, we were struggling to evaluate this function. After introducing Incucyte®, we placed immune cells and NK cells together on a plate, and made repeated observations of their behaviors using this system. This trial led to success in functional evaluation. We found that, although there was almost no change in the first 24 hours, the cells finally began to move after the second day. Such an evaluation would be difficult with other systems, because it was possible only in the incubator, and only after at least 3 to 4 days of undisturbed conditions. It was not even known how many days would be required for the immune cells to start to move, after the NK cells had secreted substances to recruit other cells. A quantitative evaluation is yet to start; however, for now it is very significant that we are at least able to evaluate the function. An evaluation of the function of NK cells to recruit immune cells and their ability to migrate to cancer cells would have been difficult to conduct with any system other than Incucyte®.

Explicit images support the long-time observation of the behavioral process

—— Is there anything else that has been discovered using Incucyte®?

Our NK cells have a transfectionally enhanced ability to attack cancer cells bound to antibodies (antibody-dependent cytotoxic activity). With Incucyte®, we became able to comprehensively evaluate both the number of cells and the types of antibodies. The use of Incucyte® has become a routine method within our company for evaluating NK cell functions when developing and producing new types of NK cells. In addition, we had previously evaluated the cytotoxic activity of NK cells for a short period of time, such as 4 hours or 12 hours. However, with Incucyte®, it is possible to observe such activity for a longer period, allowing us to continue monitoring for 4 days to a week, up to the disappearance of the cancer cells. This enables an evaluation over time. In this way, a variety of mechanisms of action have been revealed: the stage when the NK cells attack cancer cells, as well as whether the patterns of attack depend on the type of cancer. An interesting point is that NK cells may continue to attack cancer cells for the first few hours, and then stop completely. We also found that NK cells do not always act in a linear manner. For example, they may stop or resume their attack, depending on changes in the expressions of surface proteins on the cancer cells. This behavior could not have been detected if we had used short-term endpoints only. I think the ability to observe the process as a set of explicit images leads to a deeper understanding, both in terms of the growth and behavior of NK cells.

—— What is the most surprising behavior you found during close observation of NK cells?

Their non-linear manner of attack. When NK cells encounter cancer, they first attack explosively, but then calm down. Afterwards, there is the next stage of the attack, where the NK cells finally kill all of the cancer cells. I had originally thought it was simply a linear attack; so, I was surprised to learn that the initial attack was not necessarily directly related to effectiveness. This would have remained unknown had we not used Incucyte® for the evaluation. NK cells that are induced to differentiate from iPS cells may have different characteristics, even if they look the same. An evaluation of cell function over time is very important for the development of methods for manufacturing NK cells.

—— It is also important to understand that NK cells reliably kill cancer cells, even if it takes a long time.

If there are dozens of more NK cells than cancer cells, a short-term decisive battle may be acceptable. However, in reality, it is meaningful when a small number of NK cells that reach the cancer are able to steadily increase locally and attack the cancer. Therefore, cells that can continue to increase and attack for a long period of time are useful. It is also necessary to enhance their proliferative ability.

I want to create an environment where NK cells and immune cells can work together, engaging in an all-out battle.

—— What are your aspirations for the future?

Ultimately, we would like to enable the treatment of solid cancers with transfected and modified NK cells. The functions of NK cells, including CAR-NK cells, are improving, and the next challenge will be to enhance the functions of NK cells according to cancer type. In addition, even if NK cells alone cannot beat cancer, our NK cells have the function of recruiting other immune cells. If we can develop NK cells with the ability to create an environment where many immune cells are attracted and recognize the target cancer, they are expected to also be effective against cancers for which no therapies are currently available. I continue to be amazed by NK cells. For example, when NK cells are co-cultured with cancer cells that have been modified by transfection into feeder cells, the NK cells continue to increase dramatically. As long as feeder cells are present, the NK cells continue to increase at a rate of 100-fold per week. NK cells with this function can continue to increase in vitro as long as the cancer is present. If this happens in the living body, it may be possible to eradicate cancer. Of course, cancer cells are not so simple; however, I hope that one day it will be possible to treat cancer patients with our NK cells.

—— Please give a message to researchers working in similar fields and those who aspire to do so.

In the fields of drug discovery and regenerative medicine, the development of technology by imparting iPS cells with particular functions will continue to progress. As it has become possible to produce cells and tissues with functions and features that are superior to those of normal cells and tissues, there is great potential for treatment methods that have not yet been devised. Furthermore, it may also be possible to produce organs with functions that are enhanced by gene transfer. As Japan is the creator of iPS cells, we would like to do our best together with you. I expect that various iPS cell-based cells will be developed by academic institutes, companies, venture companies, and other bodies, and I hope it will become an exciting wave. I would like to tell researchers and students that we should definitely work together.

(Interview / Text: Nozomi Sakamoto)

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