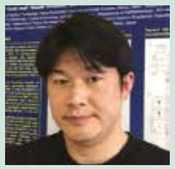
Impact Objectives

- Investigate how inhibiting T cell exhaustion can improve immunotherapy
- Research on energy metabolism and epigenetic control in T cells

A protocol for T cell immunotherapy

By exploring and better understanding the process of T cell exhaustion, ***Professor Takeshi Yamada*** *is seeking to develop treatments for refractory cancer*



How did you become interested in this area of research?

I began studying chronic infectious diseases, such as HIV and hepatitis C virus infections, as a virologist at the University of Tokyo in 1996. During that time, I learned that T cells in these patients are exhausted due to sustained antigen stimulation and other factors, which leads to T cell dysfunction against viruses. This also led me to become interested in T cell exhaustion which is problematic in refractory cancer. My turning point was when I decided to study abroad in the US. This was when I started my career as an immunologist studying CD8 T cell differentiation at Baylor College of Medicine in 2005. Since then, I have been researching exhaustion and differentiation of CD8 T cells at Ehime University (2013 to 2018) and now at Ehime Prefectural University of Health Sciences (since 2018).

What are some of the big gaps in our knowledge about the function and mechanism of the immune system that you are hoping to fill with your studies?

We are working to elucidate how T cells become dysfunctional in order to improve T cell immunotherapy by developing a method to inhibit exhaustion. In order to do this, we have been studying the mechanisms of T cell differentiation related to exhaustion through focusing on energy metabolism and epigenetic control in T cells.

Immunotherapy is a very innovative way to treat patients with refractory cancer. However, its effect is limited in some patients, partly because T cell exhaustion is induced with high expression of inhibitory receptors (known as immune checkpoint molecules) such as PD-1, LAG3 and CTLA-4. We need to know the reasons why exhaustion occurs in patients in order to improve T cell immunotherapy. This is the main focus of our efforts in our research

From your perspective, what makes your research novel in regards to suppressing T cell exhaustion during T cell immunotherapy?

We are using innovative methods that will raise the versatility of protocols against many kinds of cancers. It is a new idea to suppress T cell exhaustion and improve the quality of T cells by culturing with drugs. Our method is quite simple as it involves adding drugs that have already been used into a cell culture to control intracellular energy metabolism or epigenetic enzymes.

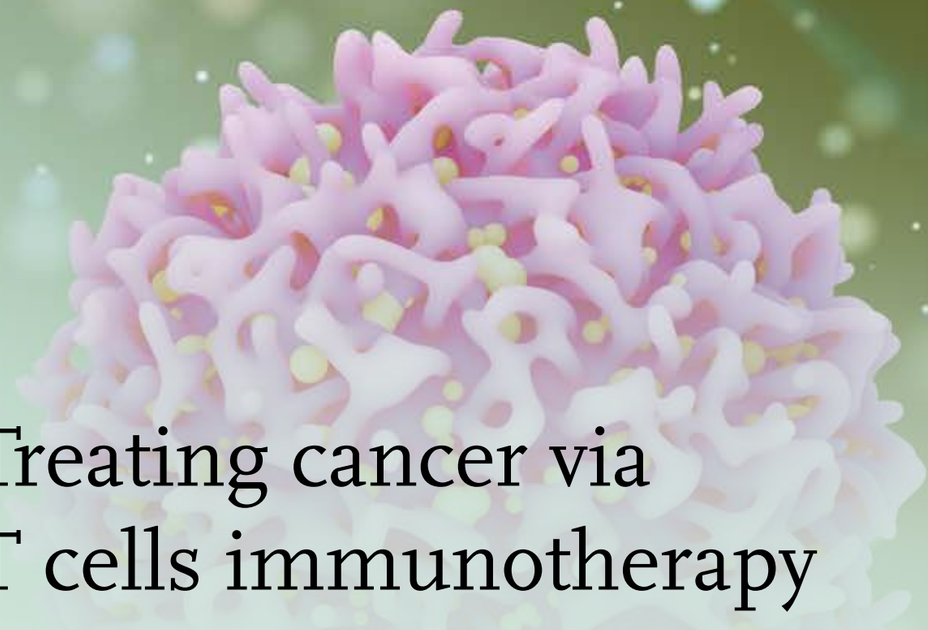
We are developing new protocols to suppress exhaustion using comparatively convenient ways such as drugs that can be added into T cell culture before adoptively transferring them to cancer patients. Our methods will be applicable to many types of cancer as long as the cause of failure of immunotherapy is due to the exhaustion of T cells. So, in the first instance, patients with refractory cancer will likely benefit from our protocols.

Can you talk a little about some of the results you have seen so far with your experiments?

Our preparative experiments have shown some positive results, including that tumour-specific T cells cultured with drugs to inhibit glutamine metabolism decreased the tumour growth and increased the survival of mice carrying tumour cells, compared to the control without drugs. These results indicate promising outcomes of the new protocols we are developing.

How are you hoping to build on what you have discovered through your research so far?

Our next steps will be to find the best conditions to culture tumour-specific T cells using drugs to control intracellular energy metabolism or epigenetic change in T cells in order to gain the largest effects on anti-cancer therapy. Next, we will focus on applying our methods from mouse model to the human body. ●



Treating cancer via T cells immunotherapy

At ***Ehime Prefectural University of Health Sciences****, researchers are exploring a revolutionary new way to treat cancer that involves suppressing T cell exhaustion during T cell immunotherapy*

Professor Takeshi Yamada has dedicated his career to this field. He is based at the Department of Medical Technology, Immunology, Ehime Prefectural University of Health Sciences in Japan where he is leading a team seeking to elucidate the mechanisms of T cell exhaustion in order to improve T cell immunotherapy. He explains that adoptive immunotherapy, in which T cells are taken from a patient and grown in a laboratory in order to increase the number of tumour-specific T cells before being returned to the patient, can be used to treat intractable cancers by increasing the number of T cells on hand to fight the cancer, thereby boosting the immune system's cancer-fighting ability. One barrier that researchers are encountering, though, is that once outside of the body, the T cells can fall into a state of exhaustion and are therefore unable to provide effective treatment. As the mechanisms of T cell exhaustion aren't well-known, this is a hurdle that needs to be overcome.

Yamada and his team want to find a way to inhibit T cell exhaustion, and their studies are focusing on intracellular energy metabolism and epigenetic control (epigenetic mechanisms regulate biological processes) in T cells using mouse models. 'We are developing protocols to improve T cell function for immunotherapy by controlling epigenetic changes involved in glutamine metabolism, which induces T cell exhaustion,' he outlines. Ultimately, they want to improve the culture method of T cells in adoptive immunotherapy in order to suppress T cell exhaustion. 'This is a novel approach, with previous research having focused on activating and proliferating tumour-specific T cells,' he says. In addition, the team's focus on epigenetic control is an innovative angle. Overall, this novel work has the potential to make significant inroads in the treatment of cancers, particularly intractable cancers.

EPIGENETIC CHANGES AND CONTROL

The researchers are interested in the process of T cell differentiation which, simply put, is when a T cell transitions from one cell type to another. They believe that T cell exhaustion occurs after excess proliferation and differentiation when there are too many nutrients and too much stimulation of antigens or cytokines in the T cell culture. 'The exhaustion must be linked with T cell differentiation and senescence by epigenetic changes via glutamine metabolites such as α-ketoglutarate,' Yamada explains.

As such, the researchers' work surrounds the mechanism of T cell differentiation via intracellular energy metabolism and epigenetic changes and how this can impact on exhaustion. 'In previous research, the team clarified that the enhancement of glutamine metabolism that occurs during the activation of T cell cultures causes epigenetic changes that induce T cell exhaustion,' states Yamada. 'Essentially, the glutamine metabolite α-ketoglutarate acid triggers the activation of a protein called histone H_3K_{27} demethylase, which induces T cell exhaustion through epigenetic changes,' he confirms. Yamada explains the science behind this ▶

Campus photo of Ehime Prefectural University of Health Sciences

and the path his research is following. 'We have reported that exhaustion is caused partially by epigenetic changes, such as activation of histone H3K27 demethylase using α-ketoglutarate as a cofactor - α-ketoglutarate is a product of glutamine metabolism,' he explains. 'We also reported that the inhibitor of glutamine metabolic enzymes or histone H3K27 demethylase reduced exhaustion of cultured T cells.' The team will apply these studies to develop new methods in order to inhibit exhaustion and improve T cell immunotherapy using drugs which restrict the activity of glutamine metabolism or histone H3K27 demethylase.

'Indeed, in our preliminary studies, the team found that adding the inhibitor of glutamine metabolism or histone H3K27demethylase to CD8 T cell cultures enhances differentiation into unexhausted long-lived T-cell as known as immunological memory,' says Yamada. They are now expanding on this finding in order to develop a method to suppress T cell exhaustion via epigenetic control. This work covers three experiments involving CD8 T cells, otherwise known as 'killer cells' for their tumour killing ability, conducted over a three-year period. 'The first of the three experiments is an exploration of the optimal culture conditions for enhancing anti-tumour activity via epigenetic control,' highlights Yamada. 'In the second experiment, the researchers will perform a detailed analysis on cell proliferation and differentiation using cultured cells and will clarify the mechanism behind enhancing anti-tumour activity,' he says. Finally, in the third experiment, Yamada and the team will look into how combination treatments with molecular drugs, that are currently used to treat cancer, can be used to further enhance the effect of epigenetic control on anti-tumour activity.

> *"We are developing protocols to improve T cell function for immunotherapy by controlling epigenetic changes involved in glutamine metabolism, which induces T cell exhaustion"*

ANTI-TUMOUR ACTIVITY

In experiment one, the researchers will culture tumour-specific CD8 T cells using the inhibitor of glutamine metabolism or histone H3K27demethylase, and anti-tumour analysis will be performed using a cancer mouse model, with the tumour size measured over time in order to verify the anti-tumour activity of the T cells. This way, the team will be able to decipher the optimum culture conditions. 'As part of experiment two, we will then collect the transferred T cells infiltrating the tumour from the mice and perform an analysis to clarify the mechanism of enhancing anti-tumour activity by adding drugs to the culture,' explains Yamada. The team also intends to perform culture experiments using T cells from humans in order to see if the same results are observed. 'In experiment three, in order to clarify the effects of combined treatment, a kinase inhibitor or monoclonal antibody drug will be administered to tumour-bearing mice that have undergone adoptive immunotherapy. Finally, the researchers will propose a new immunotherapy protocol using an epigenetically controlled culture containing histone H3K27 demethylase inhibitor,' he concludes.

This research involves the important contributions of valuable collaborators, with expertise in mouse models, translational research and T cell differentiation. 'I am collaborating with Dr Yuya Arakwa, who is an assistant professor in our team and a key person on our project. He plays a critical role in experiments and analyses using mouse models,' comments Yamada. The team are also collaborating with Dr Masaki Yasukawa and Yoshiaki Norimatsu at Ehime Prefectural University of Health Sciences on translational research for cancer patients, Dr Masakatsu Yamashita at Ehime University for important discussions on T cell differentiation, and Dr Yuki Imai in Ehime University and Dr Hirotake Tsukamoto in Kyoto University on the work with mouse models.

A LIFE-SAVING PROTOCOL

The next steps for the researchers will be to translate their mouse model findings to humans. 'We will confirm the effects of drugs using human T cells in the culture and then confirm the effects and safety of our method in the human body by injecting cultured cells,' says Yamada. 'We believe that our method is safe as it doesn't involve directly injecting drugs into the human body.' If the researchers are successful in proposing a protocol for T cell immunotherapy with higher anti-tumour effect than has been seen previously, this could lead to an effective treatment for many cancers, including those that are intractable. Ultimately, this could revolutionise cancer treatment and save lives. ●

Project Insights

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Professor Takeshi Yamada has worked at the University of Tokyo, Baylor College of Medicine and Ehime University. Yamada currently works at the Department of Medical Technology, Laboratory Technology, Immunology, Ehime Prefectural University of Health Sciences. He is a member of the Japanese Society for Immunology and The Japanese Cancer Association.