News Release

Title

A novel system to assess the safety of chimeric antigen receptor (CAR) T cell and *piggyBac* transposon-mediated CD19 CAR-T cell

Key Points

- We developed a new tagmentation-assisted PCR (tag-PCR) with the aim of assessing the safety of chimeric antigen receptor (CAR) T cell in a timely and comprehensive manner.
- We described the safety of *piggyBac* transposon, a non-viral gene transfer vector that mediates CD19 CAR-T cell.
- *PiggyBac* transposon-mediated CAR-T cell could have potential therapeutic applications, and tag-PCR represents a useful method to assess the safety of CAR-T cells.

Summary

Prof. Yoshiyuki Takahashi, Dr. Hideki Muramatsu, and Dr. Motoharu Hamada at the Department of Pediatrics, Nagoya University Graduate School of Medicine (Dean, Kenji Kadomatsu, M.D., Ph.D.), Dr. Yusuke Okuno and Nobuhiro Nishio at the Center for Advanced Medicine and Clinical Research, Nagoya University Hospital, together with Prof. Yozo Nakazawa at the Department of Pediatrics, Shinshu University, along with their colleagues developed a novel tagmentation-assisted PCR (tag-PCR) technique aimed at assessing the safety of genetically modified cells and evaluated the safety of CD19 chimeric antigen receptor (CAR) T cells using tag-PCR.

CD19 CAR-T cell therapy is a novel treatment for refractory or relapsed acute lymphoblastic leukemia (ALL) that is incurable with conventional therapy. In CAR-T cell therapy, the patient's T cells receive a CAR gene transduction and are expanded outside the body. Subsequently, expanded CAR-T cells, which can intensively and sequentially attack leukemia cells, are injected back into the patient. To date, in the majority of clinical trials of CAR-T cells globally, a viral gene transfer system (e.g., retroviral and lentiviral) has been used. However, the Department of Pediatrics at Nagoya University developed the *piggyBac* transposon, a non-viral vector system that mediates CD19 CAR-T cells. This system has higher safety and is cost effective. Genetically modified cells, including CAR-T cells, may potentially mutate and turn cancerous following oncogene activation induced by the integration of foreign genes near the oncogene. Therefore, it is necessary to evaluate their safety by inspecting integration sites of the CAR gene in CAR-T cells. To this end, we developed a new tag-PCR technique aimed at assessing the safety of CAR-T cells and evaluated the safety of CD19 CAR-T cells using tag-PCR.

Compared with conventional methods, tag-PCR could be used to evaluate the safety of CAR-T cells in a timely and comprehensive manner. Additionally, tag-PCR demonstrated that

piggyBac CAR-T cell safety was comparable to that of retroviral CAR-T cells, which have demonstrated its safety over long periods following injection in humans.

Tag-PCR is a useful technique for assessing the safety of CAR-T cells. Additionally, it can be applied to other genetically modified cells, such as those used in gene cell therapy. Based on our findings, *piggyBac* CAR-T cells represent a potential therapeutic approach for both cancer and other diseases.

Research Background

Acute lymphoblastic leukemia (ALL) is the most common hematological malignancy in children. A survival rate of over 80% is achieved using chemotherapy with or without stem cell transplantation. However, because of the poor prognosis of refractory or relapsed ALL, a new therapeutic approach is required. A novel CD19 chimeric antigen receptor (CAR)-T cell therapy represents a possible treatment for patients with refractory or relapsed ALL. In CAR-T cell therapy, the patent's T cells receive a CAR gene transduction and are expanded outside the body. Subsequently, expanded CAR-T cells, which can intensively and sequentially attack leukemia cells, are injected back into the patient. Clinical trials have demonstrated that CD19 CAR-T cell therapy is effective in 70%–90% of patients with refractory or relapsed ALL. Based on these results, this therapeutic approach has recently gained popularity. To date, CAR-T cells have been generated using viral gene transfer vectors. We have developed a novel *piggyBac* transposon, which is a safe and cost-efficient non-viral gene transfer vector that mediates CAR-T cells. Notably, phase I clinical trials of *piggyBac* CD19 CAR-T cells have recently begun in Japan. Of note, genetically modified cells, including CAR-T cells, have a potential risk of mutagenesis. In this scenario, cells turn cancerous because of oncogene activation induced by the integration of foreign genes near oncogenes (Figure 1).

To date, various methods are available to determine integration sites of genetically modified cells. However, these techniques have the drawback of being time-consuming and incomplete.

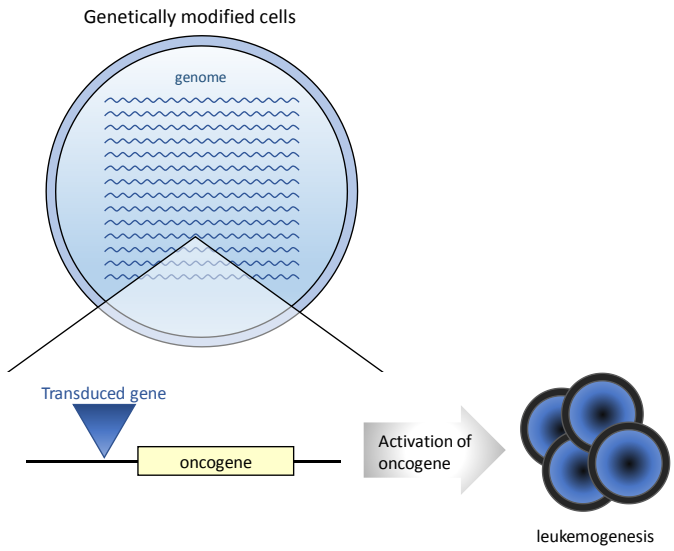

Figure 1. Malignancy mechanism of genetically modified cells

A foreign gene, integrated near an oncogene, mediates the oncogene's activation resulting in malignant transformation of the cell itself.

Research Results

We developed a novel tag-PCR technique aimed at determining the integration sites of CAR gene in CD19 CAR-T cells. Using tag-PCR, we evaluated the safety of both CD19 CAR-T cells generated by viral gene transfer system (retrovirus and lentivirus) and the *piggyBac* transposon system.

We observed that *piggyBac* transposon's integration frequency near regions associated with gene activation was significantly lower compared with the retrovirus system. Additionally, we found a comparable frequency of insertions into oncogenes for the retroviral vector. Compared with the lentivirus vector, *piggyBac* transposon integrated with higher frequency near regions associated with gene activation and with lower frequency near regions associated with integration into oncogenes (Figure 2). These results indicate that the safety of *piggyBac* CAR-T cells is comparable to that of retrovirus CAR-T cells. Specifically, the latter have a long history of demonstrated safety for genotoxicity in humans.

Tag-PCR can be used to evaluate the safety of CAR-T cells in less than half the time and steps associated with conventional methods. Furthermore, similar to conventional methods, tag-PCR can comprehensively evaluate the safety of CAR-T cells. These results show that tag-PCR could evaluate the safety of CAR-T cells in a simple and comprehensive manner. Additionally, this technique can be applied to other genetically modified cells.

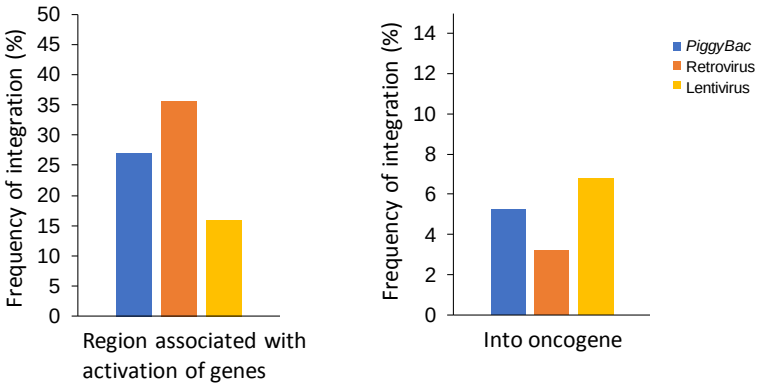

Figure 2. Frequency of integration into the region associated with gene activation and into oncogenes.

Compared with retroviral vectors, *piggyBac* transposon had a lower frequency of integration into the region associated with gene activation. Additionally, the frequency of integration into oncogenes was comparable between the two systems. Compared with lentiviral vectors, *piggyBac* had a higher frequency of integration into the region associated with gene activation; however, integration into oncogenes was lower compared with that for lentivirus.

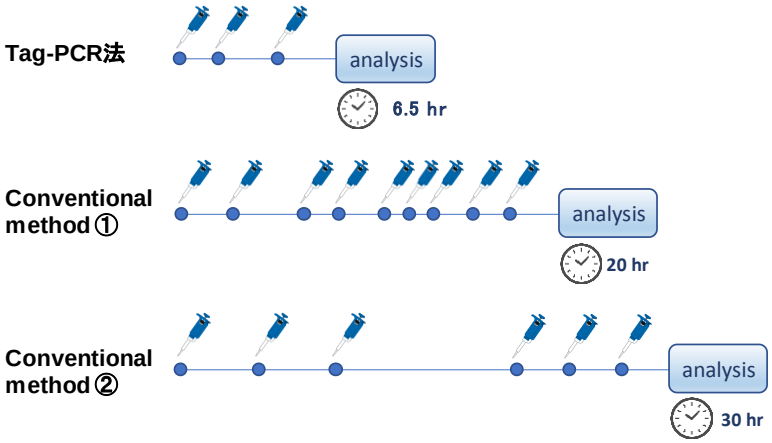

Figure 3. Time course of tag-PCR and two conventional methods.

Each blue circle and labware icon indicates an experimental step. Estimated durations are indicated beside each method. Compared with conventional methods, tag-PCR can analyze the integration sites in a more timely and simple manner.

Research Summary and Future Perspectives

Our research demonstrated that tag-PCR is a useful technique for evaluating the safety of genetically modified cells. Additionally, this technique can be applied to broader areas. Our results also demonstrated that the safety of *piggyBac* CAR-T cells was comparable to that of retroviral CAR-T cells. Considering the reduced cost of vector production, *piggyBac*-mediated CAR-T cells could represent an achievable therapeutic approach for both cancer and other diseases.

Publication

Motoharu Hamada, Nobuhiro Nishio, Yusuke Okuno, Satoshi Suzuki, Nozomu Kawashima, Hideki Muramatsu, Shoma Tsubota, Matthew H. Wilson, Daisuke Morita, Shinsuke Kataoka, Daisuke Ichikawa, Norihiro Murakami, Rieko Taniguchi, Kyogo Suzuki, Daiei Kojima, Yuko Sekiya, Eri Nishikawa, Atsushi Narita, Asahito Hama, Seiji Kojima, Yozo Nakazawa, and Yoshiyuki Takahashi

Integration mapping of piggyBac-mediated CD19 chimeric antigen receptor T cells analyzed by novel tagmentation-assisted PCR.

EBioMedicine, August 3rd, 2018

DOI: 10.1016/j.ebiom.2018.07.008

Japanese ver.

https://www.med.nagoya-u.ac.jp/medical_J/research/pdf/EBioM_20180820.pdf