R&D Item

5. Evaluating remote control of intracellular CA *in vivo*

Progress until FY2024

1. Outline of the project

In this research and development project, we will identify suitable cells to be loaded with intracellular Cybernetic Avatars (hereafter referred to as intracellular CAs), as well as their target cells, and efficiently isolate these selected cells and CA-loaded cells from mouse or human specimens. In collaboration with R&D items 2 to 4, we will work on the design and optimization of intracellular CAs, as well as the development and refinement of techniques for introducing them into cells..

(5-1)
Development of technologies for biometric information acquisition by extraction of Avatar Cells
Naotomo Tottori
Kyushu University

(5-2)
In-vivo Evaluation of Teleoperation of Intracellular CAs using Cancer and Senescent Cells
Akiko Takahashi
Japanese Foundation for Cancer Research

(5-3)
In-vivo Evaluation of Teleoperation of Intracellular CAs using Immune Cells
Satoshi Yotsumoto
Tokyo University of Pharmacy and Life Sciences

2. Outcome so far

In R&D Item 5, we will establish a highly efficient method for isolating cells targeted for CA (Cybernetic Avatar) integration and CA-loaded cells, in order to enable their generation and functional evaluation (5-1). In collaboration with R&D item 2 to 4, we will evaluate whether the intracellular CA-loaded cells exhibit effective functional controllability — such as detection, elimination, and functional shutdown — toward the target cells (5-2), (5-3) *in in vitro* settings. Furthermore, we will also conduct *in vivo* evaluations of the functional controllability of the intracellular CA-loaded cells (5-2), (5-3).

(5-1): We successfully isolated the target cells from a small amount of human samples while maintaining high cell viability, and confirmed that the isolated cells retained their proliferative capacity. Furthermore, to evaluate the function of CA-loaded cells in animal experiments, we collaborated with Section (5-3) to conduct separation tests using small volumes of mouse blood. These tests also confirmed that the target cells could be isolated with high efficiency and viability (Figure 1).

(5-2): We have initiated functional evaluation of intracellular CA by observing the three-way interaction among target cells, inspector cells, and remover cells using a cell dynamics monitoring and sorting platform, as well as single-cell RNA sequencing analysis.

(5-3): We selected immune cells, which can be readily shared and utilized across the project, as the host cells for intracellular CA integration. To enable cooperative elimination of target cells by CA-loaded cells, we designed and constructed the necessary genetic components.

Furthermore in (5-2), (5-3), using a platform for dynamic cell monitoring and separation, we evaluated the functionality of CA-loaded cells and confirmed that CA-loaded effector cells effectively eliminated the target cells (Figure 2).

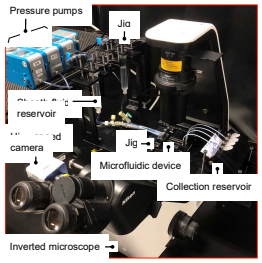

Figure 1:
The microfluidic chip designed with micropillar array contributes to the efficient and viable separation of target cells from human or mouse samples for CA loading.

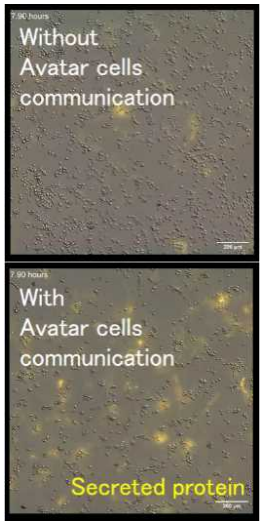

Figure 2:
After intercellular coordination, CA-loaded cells recognize target cells and cooperate in inducing cytotoxicity by secreting key proteins essential for target cell elimination. This was confirmed through analysis using a platform for dynamic cell monitoring and separation.

3. Future plans

We will evaluate whether the engineered CA-loaded cells exhibit the intended functional controllability in response to target cells, using the established experimental system and the platform for dynamic cell monitoring and separation. This evaluation will be conducted in collaboration with other R&D items, and the CA-loaded cells will be refined as needed to achieve more stable functional controllability, with the goal of enabling their functionality within living organisms in the future.