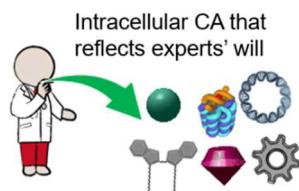


R&D Item

2. Intracellular CA designs

Progress until FY2024

1. Outline of the project



The realization of a society that is monitored by the remote control of intracellular cybernetic avatars (hereinafter referred to as “intracellular CA”) requires (1) a function to detect cells that cause physical disorders, (2) a function to amplify the discovered information, (3) a function to convert the amplified information so that it can be detected outside the body, (4) a function to remove the causative cells, (5) a switch pathway that can drive or stop the function intracellular CA by remote control, and (6) a mechanism to grasp and achieve improvement through cooperation and coordination between cells carrying intracellular CA in the body. In this R&D Item, we will design and construct intracellular CAs with these functions above. The constructed intracellular CAs will be loaded onto the cells selected in accordance with R&D Item 5 using the technology described in R&D Item 3, and evaluated in cooperation with R&D Items 4, 5, and 6.



(2-1)
Development of Intracellular CAs with Signal Conversion Function
Maasa Yokomori
University of Tokyo



(2-2)
Development of Compound-based Intracellular CAs
Kosuke Dodo
RIKEN

2. Outcome so far

In R&D Item 2, we set up R&D Items (2-1), (2-2), and (2-3) to realize (1), (4), and (6) on Outline of the project. In the same manner, to realize (2) and (3), R&D Items (2-1) and (2-3) and (2-2) were set up. (2-3), and (2-5) would co-operate with each other to realize (5). By this a multilayered R&D Items, the challenging problem of designing and constructing intracellular CAs would be tackled. To date, we have conducted the following research.

(2-1) We have designed and constructed several nano CAs utilizing nanoparticles and DNA. We constructed an intracellular CA with a signal conversion to light (Figure 1).

(2-2) As one of the compound-based intracellular CAs, we proceeded to introduce a switch that can “stop” cells by compound treatment. As a result, we have succeeded in inducing cell death (apoptosis) promptly by compound treatment (Figure 2).

(2-3) As one of the gene-based intracellular CAs, we utilized the phytohormone receptor genes to establish a technology platform for “starting” and conditionally “branching” the function of CA-loaded cells under culture conditions, and filed a patent application (Figure 3).



(2-3)
Development of Gene-based Intracellular CAs
Shigeo S. Sugano
AIST



(2-4)
Development of Membrane Channel-like Intracellular CAs
Kan Shoji
Nagaoka University of Technology

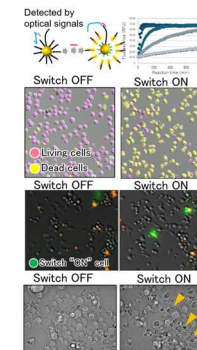


Figure 1: Example of developed intracellular CA for signal conversion using DNA and nanoparticles, a target molecule (signal) is converted to another signal (optical signal) when detected (left: schematic image, right: quantification of light signal).

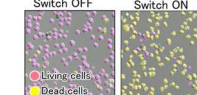


Figure 2: Remote control of cells with intracellular CA for “shutdown” The photos show cell death (apoptosis) induced in immune cells by treatment with the compound. Live (magenta) and dead (yellow) cells are marked in pseudocolor. “Shutdown” (cell death) was induced (right) when compound-based intracellular CA was processed (switch ON) for intracellular CA-loaded cells (left).



Figure 3: Remote control of cells with intracellular CA for “conditional branching.” a strain of cancer cells carrying a gene-based intracellular CA that exhibits green fluorescence under conditions of external input (remote manipulation) with phytohormones. When the intracellular CA-loaded cells (left) were turned on by the addition of phytohormone, fluorescence was observed in some cells in response (right).

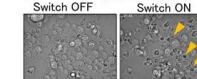


Figure 4: Forced arrest by plasma membrane channel-like intracellular CA. Photos of strain-immunocompetent cells immediately (left) and 1 hour and 45 minutes (right) after treatment with plasma membrane channel-like intracellular CA. Several cells (arrowheads) were forced to “stop” by plasma membrane channel-like intracellular CA.

(2-4) We developed a plasma membrane channel-like intracellular CA using a synthetic DNA structure as an “forced shutdown” function for CA-loaded cells. As a result, we succeeded in establishing design scheme for intracellular CAs to efficiently induce cell death by membrane channel-like structure (Figure 4).

By providing these deliverables, intracellular CAs, to R&D Items 4, 5, and 6, we have started experiments to evaluate the function of cells that have been given the function of inspection and removal.

3. Future plans

In a culture environment, we will construct multiple intracellular CAs to reflect the will of experts using designed cell-to-cell communication on immune cells or immune cells collected from human or mouse blood, and evaluate their functionality in collaboration with other R&D Items.

In addition, for the safe and secure use of intracellular CA, we will combine multiple intracellular CAs for “stopping” CA-loaded cells based on different principles and evaluate their “forced shutdown” function. For the intracellular CA for forced shutdown, we will evaluate its side-effect effects on cells.