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Okayama University research: Controlled cell death by irradiation with light

(Okayama, 31 March) **Researchers at Okayama University report in the *Journal of the American Chemical Society* the light-induced controlled death of targeted cells. The underlying mechanism involves a protein proton pump that alkalizes the intracellular environment when irradiated with light.**

In multicellular organisms, cells that are no longer needed or have become unhealthy are eliminated through a process called apoptosis — a type of programmed cell death. Apoptosis is an essential mechanism for the development and health of organisms. In many human diseases, including cancer and autoimmune disease, inappropriate apoptosis is part of the pathological picture. To better understand apoptotic cell death and devise treatments for diseases where inappropriate apoptosis plays a role, tools to stimulate or prevent cell death by means of external stimuli are nowadays being developed. Professor SUDO Yuki and Associate Professor KOJIMA Keiichi, NAKANO Shin (undergraduate student) at Okayama University has now succeeded in using light as the external stimulus to regulate apoptotic cell death. Their technique exploits the response to light of certain proteins in cell membranes.

The method of Professor SUDO and colleagues is based on the notion that the pH within a cell needs to be within a certain range for the cell to stay alive. The pH of a medium, a number between 0 and 14, is an indication of its acidity or basicity, which in turn is related to the concentration of protons. A neutral medium like water has a pH of 7. An acidic medium has a pH lower than 7; a basic or alkaline medium's pH is higher than 7.

The researchers first performed experiments with a type of human cells called HeLa cells, grown in alkaline and acidic medium. They found that the alkaline medium resulted in an intracellular pH of 8.1 and the subsequent death of the cells, whereas acidification did not. Building on this observation, Professor SUDO and colleagues grew HeLa cells with cell membranes containing proteins (rhodopsins) that can pump protons from the inside to the outside of the cell (AR3 rhodopsin) or vice versa (*RmXeR* rhodopsin). The former process lowers the proton concentration in the cell's interior, resulting in a higher pH, whereas the latter results in a lowering of the pH. Rhodopsin's pumping function is triggered by light; for HeLa cells in alkaline medium, irradiation with light during 5 minutes brought the pH to 8.6 and 7.3 for AR3 and *RmXeR* rhodopsin, respectively — accelerating and decelerating the intracellular alkalization, respectively. Similar experiments with HeLa cells in neutral medium led to similar results, showing that phototriggered alkalization initiates the apoptosis mechanism. The light-activated AR3 induced cell death at a physiologically neutral pH 7.4 and biochemical analysis revealed that the intracellular alkalization caused by AR3 triggered the mitochondrial apoptotic signaling pathway.

The scientists then checked whether phototriggered apoptosis can happen in a living organism. They irradiated worms that were genetically modified so that they produce AR3 rhodopsin in nerve cells. They found that the targeted nerve cells indeed undergo apoptosis.

The present work shows that it is possible to induce apoptotic cell death by means of irradiation with light, through the mechanism of intracellular alkalization involving the proton pump rhodopsin AR3. Regarding the relevance of this finding for new therapies, Professor SUDO and colleagues plan to "demonstrate the applicability of the phototriggered apoptosis method in tissues and organs in vivo toward therapeutic application."

Background

Microbial rhodopsins

Microbial rhodopsins, also called bacterial rhodopsins, are proteins that perform ion transport functions in bacteria, triggered by irradiation with light. They are located within cell membranes — the ion transport happens through the cell membrane, from the inside of the cell to its outside environment (outward) or vice versa (inward). The family of microbial rhodopsins contains light-driven proton pumps, ion pumps, ion channels, and light sensors. Well-known light-driven proton pumps are bacteriorhodopsin and archaerhodopsin. An archaerhodopsin known as Archearhodopsin-3 (AR3) was used by Professor SUDO Yuki and colleagues from Okayama University as an optically triggered outward proton pump. They were able to show that AR3, when irradiated with light, causes alkalization of a cell's inside environment, which in turn leads to controlled cell death (apoptosis).

Figure

A purple-colored rhodopsin, Archaerhodopsin-3, outwardly transports protons in a light-dependent manner. The intracellular alkalization induces a programmed cell death, apoptosis, in animal cells. This photo-triggered apoptosis (PTA) method has a high potential as an optogenetic tool to selectively eliminate target cells with a high spatiotemporal resolution.

Reference

Shin Nakao, Keiichi Kojima, Yuki Sudo. Phototriggered Apoptotic Cell Death (PTA) Using the Light-Driven Outward Proton Pump Rhodopsin Archaerhodopsin 3. *J. Am. Chem. Soc*. 2022, 144, 9, 3771–3775.
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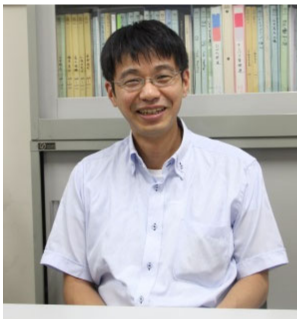

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◆About Okayama University

Okayama University is one of the largest comprehensive universities in Japan with roots going back to the Medical Training Place sponsored by the Lord of Okayama and established in 1870. Now with 1,300 faculty and 13,000 students, the University offers courses in specialties ranging from medicine and pharmacy to humanities and physical sciences.

Okayama University is located in the heart of Japan approximately 3 hours west of Tokyo by Shinkansen.

Website: http://www.okayama-u.ac.jp/index_e.html

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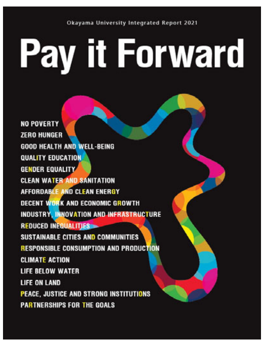

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