### Title
Photodynamic Therapy for Cancer using Mitochondrial Drug Delivery System

### Author(s)
Satrialdi

### Citation
北海道大学. 博士(薬科学) 甲第13963号

### Issue Date
2020-03-25

### Doc URL
http://hdl.handle.net/2115/77908

### Rights(URL)
https://creativecommons.org/licenses/by/4.0/

### Type
theses (doctoral - abstract and summary of review)

### Additional Information
There are other files related to this item in HUSCAP. Check the above URL.

### File Information
- Satrialdi_abstract.pdf (論文内容の要旨)
Abstract of Doctoral Dissertation

Degree requested Doctor of Pharmaceutical Science            Applicant's name Satrialdi

Title of Doctoral Dissertation

Photodynamic Therapy for Cancer using Mitochondrial Drug Delivery System
（ミトコンドリア薬物送達システムを用いた癌光線力学療法の検証）

A non-invasive and specific targeting for cancer therapy is a necessity to manifest in order to minimize the harmful effect on the non-malignant cells. During the past century, photodynamic therapy (PDT) has been actively developed as a non-invasive approach to effectively eradicate the cancer cells with minimal effect on healthy cells. The PDT effect is derived from an energy transfer reaction between light as a source of energy to molecular oxygen, mediated by a light-activated molecule, known as the photosensitizer. The dynamic interaction among these three major components in PDT produces a lethal level of reactive oxygen species (ROS), mainly singlet oxygen. The selectivity of this therapy could be achieved by the specific accumulation of the non-toxic photosensitizer in the tumor region, accompanied by the precise delivery of light in the corresponding area. The singlet oxygen has a highly reactive characteristic that can readily react with several vital molecules in the biological system, resulting in the molecule dysfunction. This interaction may also lead to irreversible oxidative damage and further provoke a lethal effect for the cells. However, the harmful effects of singlet oxygen are restricted by their short lifetime and inadequate diffusion capacity. Therefore, the specific delivery of photosensitizer, mainly in organelle-level, could be a promising strategy to obtain the maximum benefits of this therapy. Moreover, as the important organelle that holds both the vital and lethal functions, mitochondria are identified to be an attractive target for optimizing the PDT outcomes.

In the current condition, the existing photosensitizers often manifest disadvantageous features for a practical PDT application. One of them is a non-specific accumulation either in the cellular or at the subcellular level. The other drawback is the inadequate ability of most photosensitizers in absorbing near-infrared (NIR) light. The application of NIR light in PDT, especially in the optical window of biological tissues, is profoundly beneficial because NIR light has an excellent penetration ability toward tissue consisting of water and biomolecules. These problems restrict the use of such compounds in clinical applications. Therefore, the development of a novel PDT system that can fulfill the requirements of the selective organelle accumulation in combination with the long-wavelength light activation is inevitable.

The main objective of this research was to construct a novel mitochondrial targeting PDT system with the long-wavelength light activation process. To realize that goal, a synergistic combination between a π-extended porphyrin-type photosensitizer, namely rTPA, and a MITO-Porter system, a versatile mitochondrial targeting liposomal-based nanodevice, was introduced. The incorporation of the rTPA compound into the MITO-Porter system was accomplished using the hydration method with the resulting particle showed a homogenous distribution with a diameter of 157 ± 7 nm and highly positive zeta potentials of 32 ± 3 mV. This novel mitochondrial targeting PDT system, namely the rTPA/MITO-Porter, manifested a robust capacity in producing a high level of singlet oxygen, specifically in the mitochondrial compartment of tumors, during a 700-nm light irradiation process. Based on the cellular uptake and intracellular observation results, most of the rTPA-MITO-Porter particles were efficiently internalized into the cells and concentrated in the mitochondrial compartment of tumors. Furthermore, this system displayed an efficient cytotoxicity profile against two types of
human tumor cell lines, namely HeLa cells (human cervical cancer cells) and SAS cells (human squamous cell carcinoma of the tongue), as indicated by the low EC₅₀ value of 0.16 ± 0.02 μM and 0.41 ± 0.18 μM, respectively. Additionally, the apoptosis pathway was actively induced during the PDT process of the rTPA-MITO-Porter, as shown by the formation of apoptotic bodies and fragmentation of mitochondrial structure.

Inspired by the excellent cell killing ability during the *in vitro* experiments, the translation process into the *in vivo* applications has further proceeded. A slight modification on the rTPA-MITO-Porter formulation, particularly on the helper lipid composition and the total lipids’ concentration, was made without altering the mitochondrial targeting ability and the photo-induced cytotoxic capacity. The remarkable inhibition of the tumor growth was obtained in the SAS cells-bearing mouse model after a single PDT treatment of the rTPA-MITO-Porter with the rTPA dose of 8.2 μg/mouse via intratumoral administration. There was also no significant alteration on the bodyweight of the mice during the treatment, implying the promising *in vivo* cell-killing ability of this system with a high safety profile. Furthermore, the depolarization on the mitochondrial membrane was observed after the PDT process of the rTPA-MITO-Porter, indicating the damage of mitochondrial membrane due to the specific localization of the photochemical reaction on the mitochondrial compartment of tumors. Finally, the findings presented in this research serve to verify the considerable functions of the MITO-Porter system as the mitochondrial selective drug delivery technology in potentiating the PDT outcomes as well as the importance of mitochondria as the predominant subcellular target for PDT. Moreover, this novel biologically-active nanomaterial manifests an encouraging feature for PDT applications, particularly for the superficial-type cancer cells.