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ORIGINAL ARTICLES

Mitochondrial Reactive Oxygen Species and Photodynamic Therapy

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Worldwide, the number of cancer cases is increasing. Typically, they are treated by either surgery or chemotherapy. However, these treatments may be undesirable in elderly patients or those who are under medication with antiplatelet drugs. Photodynamic therapy (PDT) represents a potentially attractive treatment option for these types of patients, since it does not involve surgery and has considerably reduced side effects compared to chemotherapy. Porphyrin, one of the most commonly used photosensitizers, has the convenient property of cancer-specific accumulation and therefore, is commonly used in PDT. However, the mechanism by which this cancer-specific accumulation occurs remains unclear. We previously reported that a heme-transport protein, HCP1, was capable of transporting porphyrin compounds. HCP1 expression is associated with increased hypoxia, although the detailed mechanism by which this regulation occurs is also unknown. Here, we review available data on the mechanism of regulation of HCP1 expression through mitochondrial reactive oxygen species (mitROS). Specifically, cancer cells show increased expression of HCP1 compared to normal cells and this over-expression is reduced in cancer cells over-expressing the mitROS scavenging enzyme manganese superoxide dismutase (MnSOD). Thus we conclude that mitROS is involved in regulating HCP1 expression.

Key words: Reactive oxygen species · mitochondria · photodynamic therapy

Introduction

Despite tremendous advances in medical science, cancer remains a significant cause of mortality around the world [1)]. Traditional therapeutic approaches such as surgery, chemotherapy, and radiation therapy are still widely used to treat patients with cancer. However, these approaches have significant drawbacks, including increasing patient's physical and mental trauma and relatively low success rates. Thus, less invasive and more effective cancer therapies are required.

Photodynamic therapy (PDT) is one of a number of alternative anticancer therapies available. It uses a combination of a photosensitizer coupled with laser irradiation to generate singlet oxygen in the target tumor tissue [2-3)]. PDT treatment offers several advantages over traditional cancer therapies. First, it has a relatively low side effect profile; normal tissues do not accumulate significant amounts of photosensitizer compared to tumor tissues, so the damaging effect of singlet oxygen is restricted to the latter. Second, it does not carry a risk of hemorrhage. Recently, novel photosensitizers with improved efficiency of accumulation in tumors and hence, singlet oxygen production, have been developed to improve the efficiency of PDT [4-6)]. However, the mechanism by which photosensitizers selectively accumulate in tumors remains unclear and lack of this knowledge impedes patient confidence in this therapeutic approach. In this review, we focus on the cellular uptake mechanism of photosensitizers and the effect of PDT from the viewpoint of oxidative stress.

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Photodynamic Therapy/Diagnosis

An aging society is one of reasons for increase in cancer patient populations. Elderly patients, in particular, face significant hurdles with conventional cancer therapies, such as surgery, because of the increased risks for severe failure of organs such as heart, lung, liver, and kidney [7]. In addition, patients with cardiovascular or cerebrovascular disease, using any of the widely prescribed anti-platelet treatments, may not be good candidates for surgery. In such cases, because of its low invasiveness, PDT could be a viable treatment option for these types of patients.

In Japan, PDT is available through the public health insurance system for the treatment of early gastric, lung, and cervical cancers [8]. According to the survey of National Cancer Center in Japan, the number of patients with these diseases has been increasing in recent years. In the case of gastric cancer, the mortality rate in both males and females has increased significantly. Furthermore, lung cancer is the leading cause of death in males. In addition, the rate of cervical cancer is also increasing; the number of young female patients testing positive for papilloma virus is increasing, largely because the age at which they become sexually active is decreasing. Consequently, this leads to higher rates of cervical cancer in younger patients [9]. A standard treatment for patients with cervical cancer above Stage Ib includes whole uterus resection resulting in loss of fertility in the patient. Therefore, an effective treatment for cervical cancer that maintains a patient's fertility would be of great benefit. PDT is becoming a more attractive treatment option because of its ability to target only the affected regions of an organ and so avoid unnecessary removal of the entire organ [10].

As new photosensitizers are being developed, the PDT approach continues to improve in effectiveness [11]. Clinically, several photosensitizers for PDT have been approved: porfimer sodium (Photofrin®), which is a hematoporphyrin derivative, is used to treat early lung, gastric, esophageal, and cervical cancer in Japan [2]. In recent years, a second-generation photosensitizer [Talaporfin sodium (Laserphyrin®)] has been developed, which has reduced photosensitivity and is used for the treatment of early lung cancer and brain tumors [12]. Besides treatment, agents such as 5-aminolevulinic acid (ALA), which is a porphyrin precursor, can be used to visualize tumor areas during brain tumor resection, allowing for better prognosis [Photodynamic diagnosis (PDD)] [13].

Accumulation of Photosensitizers in Tumor Tissue

As mentioned above, photosensitizers possessing a porphyrin structure have commonly been used in the treatment of cancer patients. Cancer-specific porphyrin accumulation is one of the most important phenomena underlying the utility of PDT to affect cancer cells in a selective manner. The mechanism by which this phenomenon occurs has been investigated and, based on this, new photosensitizers have been proposed. One example is glycoconjugated porphyrin **(Figure 1)**. Cancer cells are well known to utilize glucose as a preferential fuel and often have incredibly high rates of glycolysis [14]. Thus, cancer cells express various glucose transporters and avidly take up glucose [15-16]. Based on this, glycoconjugated porphyrins derivatives can be specifically targeted to cancer cells, using glucose transporters as an uptake mechanism [17]. It should, however, be pointed out that the reason why porphyrins without sugar chains are specifically transported into cancer cells is unclear but it does suggest that multiple uptake pathways exist.

Indeed, several theories have been advanced to propose how cancer cells incorporate porphyrins. Laura Polo *et al.* reported that a binding complex between low-density lipoproteins (LDL) and porphyrins was transported into cells through the LDL receptor [18]. In a previous study, we also demonstrated that porphyrin could be transported into cells by a proton-coupled folate transporter called heme carrier protein 1 (HCP1), also known as SLC46A1 [19-20]. HCP1 was originally discovered as a heme-transport protein. Since heme has a porphyrin-based structure and

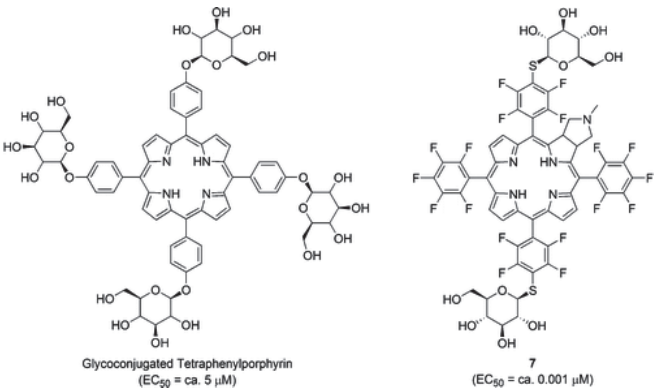

Figure 1: The structure of a glycoconjugated porhyrin which was developed for PDT (Reference #6)

adopts a conformation almost identical to that of porphyrin, HCP1 can transport not only heme but also porphyrin. In fact, uptake of porphyrin has been shown to be increased in HCP1 over-expressing cells and, likewise, is decreased in HCP1 knock-down cells supporting the notion of porphyrin transport into cells by HCP1 [20]. It has also been reported that HCP1 expression is regulated by hypoxia [21]. Hypoxia increases the generation of mitochondrial reactive oxygen species (mitROS) [22-23] and these in turn enhance the expression of HCP1, depending upon the precise ROS concentration. Transcription factors and cytokines responsive to oxidative stress responsive have been also suggested to be important in regulating HCP1 expression.

Oxidative Stress and Mitochondrial Reactive Oxygen Species (mitROS)

Eukaryotic cells synthesize ATP by oxidative phosphorylation. This aerobic metabolism is performed by the mitochondrial electron transfer system in which free electron leakage sometimes occurs allowing the generation of mitROS [24]. ROS that are generated in mitochondria are mainly superoxide anions, although other species of ROS such as the hydroxyl radical and hydrogen peroxide can also be generated [25-26]. ROS are ubiquitously generated in the body and are the principal cause of oxidative stress. They have been proposed to be involved in a variety of diseases including vascular diseases, Alzheimer disease and carcinogenesis, as well as accelerated aging [27-29] **(Figure 2)**. To protect against these diseases, the body has various defense mechanisms that act to scavenge ROS in order to suppress oxidative stress. Glutathione and glutathione peroxidase convert hydrogen peroxide to water, and superoxide dismutase (SOD) decomposes superoxide to hydrogen peroxide [30]. SOD has a metal iron at its active center and several different types of SOD enzyme have been characterized based on the active metal center, e.g., Cu- and Zn-SODs in the cytoplasm and Mn-SOD in the mitochondria [31]. In a previous study, we demonstrated that cancer cell specific mitROS enhanced cellular invasion [32]. Therefore, Mn-SOD, which predominantly scavenges mitochondrial ROS, is an important enzyme for biophylaxis.

In addition to the activity of the mitochondrial respiratory chain, other exogenous factors also promote the generation of ROS. For examples, excessive intake of salt and alcohol intake have been reported to be involved in the induction of gastric cancer [33]. We have also reported that salt and alcohol inhibit the mitochondrial electron transport chain and accelerate the generation of mitROS in gastric epithelial cells [34-35]. Furthermore, infection of the gastric mucosa with *Helicobacter pylori* also induces mucosal inflammation via the production of ROS, and is related to the resultant gastric carcinogenesis [36]. Therefore, ROS, especially mitROS, are also induced by a variety of exogenous factors and could be associated with the onset and establishment of cancer. Moreover, the over-production of mitROS may activate many signal transduction pathways, followed by the induction of proinflammatory cytokines and activation of a variety of transcription factors such as NF-κB and hypoxia inducible factor 1α (HIF-1α) [26, 37-38]. These cytokines and

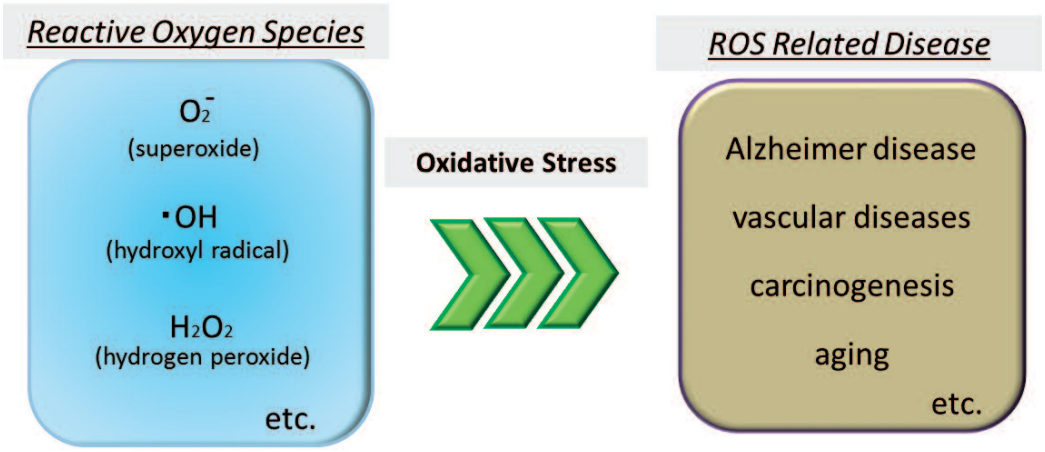

Figure 2: Schematic illustration of the relationship between reactive oxygen species and diseases.

transcription factors are related to carcinogenesis and form part of the cancer-specific phenotype. Consequently, signal transduction via mitROS may play an important role in cancer cell-specific HCP1 expression.

Effect of Mitochondrial Reactive Oxygen Species for Photodynamic Therapy

We compared the expression of HCP1 induced by different levels of mitROS, and then studied the differences in both cellular porphyrin accumulation and the PDT effect in normal and cancer cells, and cancer cells over-expressing MnSOD [39]. Generally, cancer cells showed a higher concentration of ROS than normal cells. In addition, Mn-SOD over-expression suppressed ROS generation in cancer cells [32]. We confirmed that, as expected, HCP1 was expressed at higher levels in cancer cells compared to normal cells, and that Mn-SOD over-expression in cancer cells suppressed HCP1expression [22]. The higher level of HCP1 expression in cancer cells induced porphyrin accumulation into these cells and this accumulation of porphyrin was suppressed in Mn-SOD-overexpressing cancer cells: PDT effects correlated with porphyrin accumulation levels. These results indicate that over-expression of mitROS in cancer cells enhances the PDT effect **(Figure 3)**. Based on this result, we hypothesized that promotion of ROS generation through inhibition of the mitochondrial electron transfer system would enhance the effect of PDT. We previously reported that indomethacin (IND) enhanced the generation of ROS in isolated mitochondria derived from gastric epithelial cells [40]. Non-steroidal anti-inflammatory drugs (NSAIDs), including IND, are usually prescribed as analgesics, and notoriously cause gastrointestinal injury by suppressing prostaglandin production through inhibition of cyclooxygenase. Damage to the intestinal mucosa is caused not only by components of digestive juice, such as gastric acid, but also by the ROS generated in response to the NSAIDs [41-42]. We demonstrated that administration of IND accelerated both the generation of mitROS and the subsequent PDT effect. Through this phenomenon, we were able to confirm the cancer-specific enhancement of HCP1 expression. We conclude that mitROS generated in response to IND enhanced the effect of PDT.

In a previous report, we studied the relationship between tumor malignancy and HCP1 expression in brain tumor specimens. The level of HCP1 expression was found to coincide with the malignancy of the tumor cells [43]. Moreover, since mitROS are strongly associated with the malignant transformation of cells, the acceleration of cellular malignant transformation and HCP1 expression might occur as a result of morphological changes in cells brought about by mitROS. As another example, cancer-specific mitROS generation increased the expression of peptide transporter 1

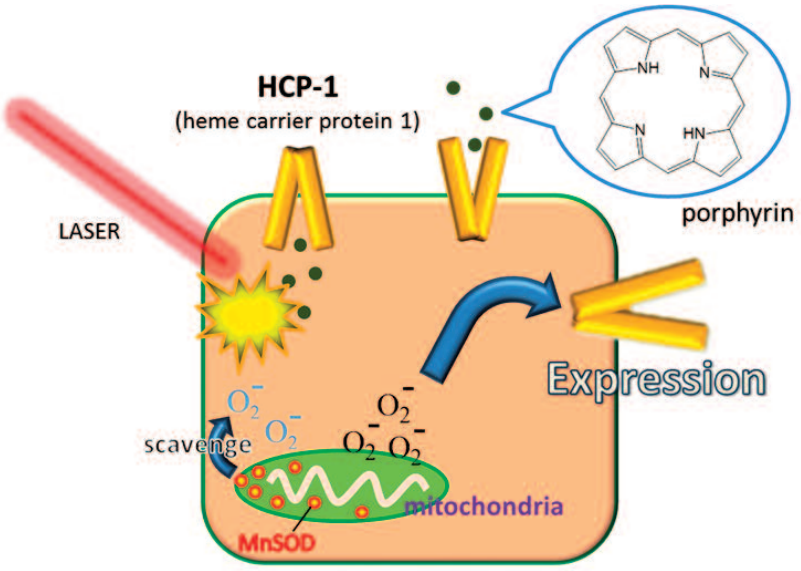

Figure 3: Schematic illustration of the expression mechanism of porphyrin-transport protein, HCP1. Over-generation of mitochondrial reactive oxygen species enhanced HCP1 expression and subsequent PDT effect.

(PEPT1), which is a transporter of ALA, to enhance the effect of PDT [44]. Accordingly, cancer-specific mitROS are likely to convert cancer cellular phenotypes via signal transduction, and enhance the effect of PDT effect by accelerating the expression of various transporters for photosensitizers.

Conclusion and future aspects

In this mini review, we have discussed data that show that the production of mitROS can improve the effectiveness of PDT, and further clarified that mitROS does so by increasing the expression of cellular transporters that are capable of transporting photosensitizing agents. Although we have focused on the cancer-specific porphyrin accumulation mechanism, many aspects of the mechanism remain to be elucidated. As mentioned above, mitROS can activate many signal transduction pathways, which in turn induce the activation of transcription factors and therefore the expression of target proteins. However, this mechanism is not always decided centrally. Furthermore, although in this paper we have focused on the uptake of porphyrin, knowledge about other aspects of porphyrin biology, such as its excretion and degradation, could also be of importance. For example, it has been reported that ROS production and the subsequent activation of HIF-1 are related to the expression of a porphyrin excretion transporter ABCG2 [45-46]. Further, the association with nitric oxide, which is a gas mediator cell signaling molecule, should be studied further.

In the near future, as the population ages and dietary habits change, a simpler and more effective treatment for cancer will be required. PDT offers one such potential approach and we are hopeful that further studies will bring this potential to fruition.

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