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Induction of hypoxia-inducible factor-1α overexpression by cobalt chloride enhances cellular resistance to photodynamic therapy

Zhenyu Ji [a,b], Guanrui Yang [b], Susan Shahzidi [a], Kinga Tkacz-Stachowska [a], Zhenhe Suo [a], Jahn M. Nesland [a], Qian Peng [a,c,*]

[a] *Department of Pathology, The National Hospital-The Norwegian Radium Hospital, University of Oslo, Montebello, 0310 Oslo, Norway*
[b] *Henan Institute of Medical Sciences, Medical College of Zhengzhou University, Zhengzhou 450052, China*
[c] *State Key Laboratory for Advanced Photonic Materials and Devices, Fudan University, Shanghai, China*

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Abstract

Although photodynamic therapy (PDT) has been approved by regulatory agencies worldwide for the treatment of several oncologic and non-oncologic conditions, PDT-induced tissue hypoxia as a result of vascular damage and photochemical oxygen consumption limits the efficacy of this modality. This may largely be due to hypoxia-mediated angiogenesis via hypoxia-inducible factor-1α (HIF-1α), a major transcription factor involved in angiogenesis, hematopoiesis and anaerobic energy metabolism. We hypothesized that hypoxia-induced HIF-1α overexpression may also lead to tumor cells resistant to PDT by favouring tumor cell proliferation. Human esophageal normal Het-1A and tumor KYSE-70 and KYSE-450 cell lines were used in the present study. High-expression of HIF-1α induced in vitro by cobalt chloride ($CoCl_2$)-mediated chemical hypoxia mimic was clearly seen in the Het-1A cell line. In addition, cells treated with $CoCl_2$ were more resistant to 5-aminolevulinic acid (ALA)-mediated PDT than those without $CoCl_2$ treatment. The photosensitivity of the cells to ALA–PDT decreased with increasing HIF-1α expression by enhancing $CoCl_2$ concentrations. Moreover, transfection of the cells with anti-HIF-1α short interfering RNA (siRNA) knocked down the HIF-1α expression and restored the photosensitivity of the cells to ALA–PDT. However, the induction of HIF-1α expression by $CoCl_2$ was not indicated in both KYSE-70 and KYSE-450 cell lines, and no difference in cell survival was found after ALA–PDT in the presence and absence of $CoCl_2$. We thus conclude that high-expression of HIF-1α induced by $CoCl_2$ plays an important role in the resistance of the Het-1A cells to ALA–PDT. The present finding suggests that hypoxia-induced HIF-1α overexpression attenuates PDT efficacy through probably not only angiogenesis, but also cellular resistance to the modality. PDT in combination with anti-HIF-1α treatment may thus enhance the PDT efficacy.

Keywords: PDT; Hypoxia; Cobalt chloride; HIF-1α; siRNA; Apoptosis

1. Introduction

Photodynamic therapy (PDT) is a promising modality of cancer treatment that utilizes tumor-specific accumulation of a photosensitizer, followed by irradiation with light at an appropriate wavelength [1]. In the presence of oxygen, the light-activated photosensitizer in tumor can generate singlet oxygen and other oxygen radicals that can react with biomolecules to cause apoptosis and/or necrosis of tumor cells [1–3]. Although PDT with endogenous protoporphyrin IX (PpIX) derived from 5-aminolevulinic acid (ALA) has been successfully applied to the treatment of several malignancies and premalignancies

* Corresponding author. Tel.: +47 22935553; fax: +47 22934832.
E-mail address: qian.peng@labmed.uio.no (Q. Peng).

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[1,2], tumor recurrence is still the major limitation of the modality [4].

Hypoxia, a common feature of solid tumors, can reduce responses of tumor cells to several cancer treatments. The effect of hypoxia on tumor cells resistant to the treatment modalities is mediated by hypoxia-inducible factor-1 (HIF-1) [5]. HIF-1 is a heterodimer consisting of HIF-1α and HIF-1β subunits, both of which are transcription factors. HIF-1β, a nuclear protein also known as the aryl hydrocarbon receptor nuclear translocator, ARNT, is constitutively expressed and independent of oxygen tension; while HIF-1α is a cytoplasmic protein responsive to oxygen level. In normoxic cells HIF-1α is continuously degraded, whereas under hypoxic conditions it translocates to the nucleus and heterodimerizes with HIF-1β to form the active HIF-1 protein, binding and activating hypoxia responsive genes like vascular endothelial growth factor (VEGF), growth factors and glycolytic enzymes [5]. Activation of these target genes serves as compensatory mechanisms allowing tumor cells to survive or escape from their hypoxic stress [6].

Several reports have recently demonstrated that PDT can induce expression of HIF-1α and VEGF as a result of hypoxia due to vascular damage as well as to photochemical consumption of oxygen [7,8]. Further, antiangiogenic treatment can significantly enhance PDT efficacy of tumor [7], suggesting that PDT induces angiogenesis, one of the possible reasons for tumor recurrence after the treatment. However, a direct effect of PDT-induced HIF-1α expression on tumor cell proliferation with resistance to the modality has not been addressed yet. In this communication, we employed cobalt chloride ($CoCl_2$), a well-known chemical inducer of HIF-1α, to study the effect of HIF-1α expression on ALA–PDT efficacy in human esophageal normal cell line (Het-1A) and squamous cell carcinoma cell lines (KYSE-70 and KYSE-450) in vitro.

2. Materials and methods

2.1. Chemicals

5-Aminolevulinic acid (ALA) was provided by Photo-Cure ASA (Oslo, Norway). Cobalt chloride ($CoCl_2$) was obtained from Sigma (St Louis, MI). The short interfering RNA (siRNA) against human HIF-1α and the mock siRNA with no match to any eukaryotic sequences were purchased from Santa Cruz Technology (Santa Cruz, CA). All other reagents were of the highest analytical grade available.

2.2. Cell culture and $CoCl_2$ treatment

Three human esophageal cell lines of Het-1A, KYSE-70 and KYSE-450 (all from ATCC) were used in the present study. Het-1A is a non-cancerous epithelial cell line immortalized by transfection with plasmid pRSV-T containing the RSV-LTR promotor and SV40 T-antigen [9]; while KYSE-70 is a poorly differentiated squamous cell carcinoma cell line and KYSE-450 is a well differentiated squamous cell carcinoma cell line. All the three cell lines were grown in RPMI 1640 medium (PAA Laboratories GmbH, Austria) supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin and 100 μg/ml streptomycin (GIBCO, Invitrogen) in a humidified incubator (Nuaire, US Autoflow) with 5% CO_2 at 37 °C. The cells were subcultured three times every week to keep the exponential growth.

Cells grown to 80–90% confluence were trypsinized and counted. Cells of 4×10^5 were seeded into 6-well plates followed by incubation with various concentrations of $CoCl_2$ for 4 h in 4 ml of serum-free medium or 24 h in medium with serum.

2.3. Western blot analysis

Cells treated with $CoCl_2$ were scraped and centrifuged. After washing once with cold PBS, cell pellets from three parallels were pooled and resuspended in 0.5 ml of lysis buffer (20 mM Tris–HCl, pH 7.2, 5 mM EDTA, 5 mM EGTA, 10 mM sodium pyrophosphate, and 0.4% SDS). After keeping on ice for 30 min, cells in lysis buffer were sonicated for 15 s and then kept at −70 °C until further analysis. Total proteins were quantified by the Bradford method using the BCA protein assay kit (Pierce, Inc., Rockford, IL) according to the protocol. These samples were boiled at 100 °C in loading buffer for 5 min and kept on ice for another 5-min. After centrifuged at 14,000 rpm for 5 min, 30 μg of each sample were electrophoresed on a 8% of SDS-polyacriylamide gel, and the fractionated proteins were then transferred to PVDF membranes by semi-dry transfer apparatus (Bio-Rad, USA). Ponceau S staining was performed on the membrane to make sure that the proteins were transferred to the membranes. The membranes were washed with PBS-Tween and blocked with 5% fat-free milk in PBS-Tween for 1 h at room temperature, and incubated with a rabbit polyclonal antibody of HIF-1α (Santa Cruz, 1:500 dilution in a 5% fat-free milk solution) at 4 °C overnight. After washing three times, the membranes were incubated for 1 h with anti-rabbit horseradish peroxidase-conjugated secondary antibody (1:2000 dilution) and finally visualized using a chemiluminescence detection kit ECL-PLUS (Amersham Biosciences). The revelation of the β-actin with an anti-β-actin antibody (Santa Cruz, 1:400 dilution) allowed to reconfirm the total amount of protein loaded on the gel.