Cell Death of Colorectal Cancer Stem-Like Cell Was Induced by Photodynamic Therapy with Protoporphyrin IX

Ming-Feng Wei[1], Shiang-You Han[1], Shu-Jyuan Yang[1], Feng-Huei Lin[1], Shih-Chieh Hung[2], Ming-Jium Shieh[1]

[1]Institute of Biomedical Engineering, National Taiwan University, Taiwan, [2]Institute of Clinical Medicine, National Yang-Ming University, Taiwan

Introduction

In recent years, the hypothesis of cancer stem-like cells (CSCs) received striking attention since many CSCs have be isolated from solid tumors, including breast cancer, prostate cancer, and colorectal cancer (CRC). This hypothesis indicated CSCs possess stem-cell-like properties, the ability to self-renew and differentiate, and induce the recurrence of tumors after chemotherapy or radiotherapy. Therefore, new therapeutic strategy must be adopted to eradicate CSCs to achieve a cure of cancer. Here, the extermination of CSCs in CRC was attempted by inducing cell death of CSCs via the use of Photodynamic therapy (PDT).

Background

Increasing evidence has suggested that a small subpopulation of cancer cells may drive the growth and progression of the tumor. Accordingly, these malignant cells that are responsible for tumour development and sustain tumor growth have been termed "cancer stem-like cells" (CSCs). Recent investigation indicated colorectal cancer stem cells have been isolated from colorectal tumor and they are characterized by the expression of CD133, a surface marker commonly found on stem cells in various tissues. It has been proposed that CSCs have resistant properties to chemotherapy and radiotherapy. Therefore, it is necessary to find new therapeutic tools for eradication of CSCs.

Photodynamic therapy (PDT) is a promising therapeutic approach for various cancers and other diseases. It is a light-activated treatment that requires administration of a photosensitizer (PS) and illuminates tumor with visible light matching the wavelength of the absorption peak of the PS. The excited PS molecules can transfer their energy to oxygen molecule and result in the formation of reactive oxygen species (ROSs) such as singlet oxygen or free radicals, lead to subcellular damage at sites where the PS accumulates and in consequence induces either apoptosis or necrosis.

Intracellular generation of the PS, protoporphyrin IX (PpIX), a heme precursor, is applied in photodynamic diagnosis and PDT of cancer. It has been reported that the accumulation of PpIX depends on key enzymes of porphyrin biosynthesis such as ferrochelatase in the tumor. Besides, from previous studies, δ-aminolevulinic acid (ALA) also causes massive accumulation of PpIX in cancer cells.

It has been demonstrated that PDT can result in an increase in expression of vascular endothelial growth factor (VEGF). The induction of VEGF is an essential component of tumor survival and growth.

Purpose

Since CSCs were resistant to chemotherapy and radiotherapy, it is in instant need of effective therapeutic strategy. So far the ability to kill CSCs with PDT has not been addressed yet. We aimed to investigate whether PDT may be an effective therapeutic tool to eradicate CSCs.

Methods

Because CD133 has been identified as a cancer stem cell marker, we utilized FACS sorting to isolated CD133-positive cells and CD133-negative cells from primary cultured cells (CCS) and HT29 cell line of colorectal cancer. Each cell subclone was incubated in darkness for 3 hours with 1 μg/mL PpIX and the intracellular uptake of PpIX was quantified using a multi-mode microplate reader with excitation and emission wavelength of 405 and 632 nm, respectively. Moreover, the cells treated with PpIX were exposed to the red light generated by the LED (632 nm) with various light-dose for photodynamic treatment. The viability of cells after treatment with PpIX-mediated PDT was measured by the Premixed WST-1 Cell Viability Assay. In addition, the VEGF production by each cell subclone was quantified from each sample using human VEGF-A ELISA kit after PDT.

Results

In this study, by FACS sorting the high percentage (> 80%) of CD133-positive subpopulation was isolated in CCS cells and HT29 cell line. For PS uptake, the amount of PpIX uptake in CD133-positive cells was approximately four-time-higher than that in CD133-negative cells. Moreover, the cytocidal effects of PDT were evaluated for each cell subclone. The PpIX-mediated PDT treatment (5 J/cm^2) could induce most of CD133-positive cells to apoptosis. In addition, CD133-positive cells could secret more VEGF than CD133-negative cells after PpIX-mediated PDT.

Conclusions

Our data showed that CD133-positive cells take up significantly more PpIX than CD133-negative cells. Although the cytotoxic effect of PDT in CD133-positive subpopulation (approximately 20% survival at 5 J/cm^2) is lower than CD133-negative subpopulation (approximately 20% survival at 2 J/cm^2), the sensitivity of CD133-positive subpopulation to PDT is still striking. Additionally, our study indicated that the VEGF production by CD133-positive cells is more than that by CD133-negative cells after PpIX-mediated PDT. Based on these results, we proposed that PDT may be an effective therapeutic tool to eradicate CSCs. Furthermore, understanding of response by CD133-positive cells following PDT will help design a new and more effective PDT treatment.