DOCTORAL THESIS

Photodynamic therapy in nasopharyngeal carcinoma: mechanistic studies of five photosensitizers

Yow Li, Miu Ngan

Date of Award:
2003

Photodynamic Therapy in Nasopharyngeal Carcinoma : Mechanistic Studies of Five Photosensitizers

YOW Li Miu Ngan

A thesis submitted in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

Principal Supervisor : Dr LEUNG Wing Nang

Hong Kong Baptist University

January 2003

Abstract

Photodynamic therapy (PDT) is an FDA approved therapeutic modality for cancers and a number of diseases. It is based on the administration of an exogenous photosensitizer (PS), activated by specfic wavelength light irridation and generating reactive oxygen species for preferential tumour eradication. Current research in PDT aims at elucidating the photodynamic mechanisms of cell death with better photosensitizers.

The purpose of this study was to explore the photodynamic potential of five photosensitizers of different nature for nasopharyngeal carcinoma cells (NPC) using the well-differentiated NPC/HK1 and the poorly differentiated NPC/CNE2 cell line as models. First of all, the efficacy for the five PS, hematoporphyrin derivative (HPD), MC540, mTHPC, ALA and ALA-methyl ester endogenously stimulated PpIX were comparatively studied for their phototoxicity, dark toxicity, cellular drug uptake kinetics, intracellular localization. The possible genotoxic damage which might induce DNA damage under dark and sub-lethal light dose (less than LD_{25}) in the environment were also investigated.

Among those studied photosensitizers, mTHPC was identified as the most superior drug for NPC photoactivation. Subsequently, mTHPC was extensively studied by delineating the underlying cell death mechanisms for both cell lines and data were published. mTHPC localises in the mitochondria of both cell lines. Upon irridation, it elicited a mitochondria-mediated apoptotic cascade. A series of apoptotic pathway events in mTHPC-PDT were undertaken sequentially. These involved phosphatidylserine (PS) redistribution, cytochrome c release, alternation of bcl-2 apoptotic family protein expression and eventually led to the DNA fragmentation. Furthermore, the pre- and post mTHPC-PDT regulation of metastatic extracellular matrix protein, MMP-2 and MMP-9 activity was also evaluated.

Sumarising the results, mTHPC-PDT was found targeting the mitochondria and produced striking damage in these organelles as revealed by the morphological changes such as cell shrinkage, membrane blebbing, chromatin condensation, cristae disruption captured under the light microscopy and transmission electronic microscopy (TEM). The photodamage were drug dose-, light dose- and cell line dependent. Thereafter, some key cellular events changes occurred. Phosphatidylserine (PS) membrane redistribution was designated as one of the hallmark of early apoptosis and was obviously shown here. Cytochrome c release was examined by confocal laser scanning microscope (CLSM) and then quantitated by flowcytometry. The release of this mitochondrial protein increases with treatment time. Evidently, western blot analysis demonstrated a decrease in bcl-2 expression after mTHPC-PDT in both cell lines. Bcl-2 is an anti-apoptotic protein and photodamage enhanced apoptotic response. Finally, DNA fragmentation via apoptosis was detected and assessed by flowcytometry using the TUNEL assay. The percentage of apoptotic cells detected was more pronounced in NPC-HK1 cells than in NPC-CNE2 cells.

The pioneer study on Matrix Metalloproteinase-2 (MMP-2) was reported here for the first time that MMP-2 was down-regulated post-mTHPC-PDT on both NPC cell lines. This implied that mTHPC-PDT can regulate tumor metastasis and MMP-2 and is a good indicator for PDT efficacy. Photoactivation of ALA-PDT on NPC undertaken were also a new initiative and results were promising. Furthermore, there is no DNA genotoxic damage effect in any of the five photosensitizers at dark and sublethal condition by the Comet electrophoresis assay.

In conclusion, this study has confirmed and offered new evidence and insights for potential PDT treatment for NPC especially employing photosensitizing drugs, mTHPC and ALA. It is of new prospect that this novel modality can be applied in clinical setting in the near future.

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