DOCTORAL THESIS

Role of epidermal growth factor receptor (EGFR) and mitogen-activated protein kinases (MAPKs) signaling pathways in Zn-BC-AM photodynamic therapy-induced apoptosis of the well-differentiated nasopharyngeal carcinoma cell

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Role of Epidermal Growth Factor Receptor (EGFR) and Mitogen-activated Protein Kinases (MAPKs) Signaling Pathways in Zn-BC-AM Photodynamic Therapy-induced Apoptosis of the Well-differentiated Nasopharyngeal Carcinoma Cells

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ABSTRACT

Nasopharyngeal carcinoma (NPC) has a high prevalence in Hong Kong, southern China and Southeast Asia. Due to the complexity of the disease, new treatment methods for locoregionally advanced or metastatic NPC are currently under development. Photodynamic therapy (PDT), a recently developed method for effective treatment for cancer, may be used as an alternative to treat the recurrent and advanced NPC. In the present study, the biological actions of Zinc (II) 2,3,8,8,12,13,17,18-octaethylbenzochlorin amidinium salt (Zn-BC-AM) PDT on the well differentiated NPC cells were investigated.

Zn-BC-AM PDT was found to induce irreversible cell death of the well differentiated HK-1 NPC cells. Zn-BC-AM PDT was found to activate the intrinsic apoptotic cell death pathway and the sequence of activation included the induction of mitochondrial membrane potential depolarization, externalization of phosphatidylserine, proteolytic cleavage of caspase-9 and -3, chromatin condensation and formation of apoptotic cells. The expression of anti-apoptotic proteins Bcl-2/ Bcl-xL was decreased immediately after Zn-BC-AM PDT treatment while the expression of pro-apoptotic proteins Bax/ Bid was not affected. Addition of singlet oxygen ($^1O_2$) scavenger L-histidine or overexpression of Bcl-2 was found to reduce Zn-BC-AM PDT-induced apoptosis, indicating that the apoptotic process was initiated through the generation of $^1O_2$ and Bcl-2 appeared to play a cyto-protective role in retarding the cell death process.

Combination therapy with anti-epidermal growth factor receptor (EGFR) drug (or anti-EGFR antibody) and chemoradiotherapy is emerging as a novel approach for treatment of advanced NPC patients. We hypothesized that inhibition of EGFR signaling pathway might also increase the efficacy of Zn-BC-AM PDT in HK-1 cells. It is found that EGFR, Akt and ERK were constitutively activated in HK-1 cells and the activities could be inhibited by EGFR inhibitor AG1478. Inhibition of EGFR, Akt or ERK by their specific inhibitors was found to enhance Zn-BC-AM PDT-induced formation of apoptotic cells and reduced the expression of Bcl-2. The results indicate that the efficacy of Zn-BC-AM PDT may further be increased through the inhibition of EGFR signaling pathways in NPC cells.

Mitogen-activated protein kinases (MAPKs) are a group of protein kinase highly sensitive to oxidative stress. The role of MAPKs in Zn-BC-AM PDT-induced HK-1 cell death was also investigated. Zn-BC-AM PDT was found to induce persistent activation of p38 MAPK and JNK, while ERK was transiently activated after PDT treatment. Inhibition of JNK with SP600125 had no effect on Zn-BC-AM PDT-induced cell death. Transient genetic knock down of specific p38 isoforms with siRNA revealed that inhibition of p38β but not p38α nor p38δ would increase Zn-BC-AM PDT-induced cell death and apoptosis. Zn-BC-AM PDT-activated p38β appears to play a role to counteract the PDT-induced cell death.

Pro-inflammatory cytokines and chemokines produced by PDT-treated tumour have been implicated to play a role in the indirect tumour destruction. Using ELISA method, we found that PDT induced the production of IL-1α and IL-1β. In contrast, IL-8 (a cytokine with both neutrophil chemotact and angiogenic activities) was

downregulated in HK-1 cells at 24 hours after Zn-BC-AM PDT, suggesting that Zn-BC-AM PDT might indirectly reduce tumour growth through the reduction of tumour angiogenesis.

In conclusion, multiple signaling pathways are involved in Zn-BC-AM PDT-induced apoptosis of HK-1 cells. Combination therapy with Zn-BC-AM PDT and EGFR/MAPKs inhibitors may further be developed for the treatment of advanced NPC.

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