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著者(和文)	Lai Hung Wei
Author(English)	Hung Wei Lai
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論 文 要 旨

THESIS SUMMARY

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学生氏名： Student's Name	Lai Hung Wei		

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指導教員（主）： Academic Supervisor(main)	小倉 俊一郎
指導教員（副）： Academic Supervisor(sub)	

要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Aminolevulinic acid-based photodynamic therapy (ALA-PDT) is gaining more and more attention as a new alternative cancer therapy, particularly the conventional chemotherapy, due to its high efficiency and extremely low side effect nature. ALA-PDT is highly tumour-specific, where it increases the accumulation of protoporphyrin (PpIX) in mitochondria after administration of exogenous ALA, which would trigger phototoxicity of cancer cells but not in normal cells. However, PpIX, a product of ALA in heme synthesis pathway, was recently found to accumulate in normal cells, causing unnecessary cytotoxicity to surrounding healthy cells. Therefore, identifying the transporters involved in ALA uptake in both normal and cancer cell lines is important in maintaining the effectiveness and improve specificity of ALA-PDT. Peptide transporter 1 (PEPT1), proton coupled amino acid 1 transporters (PAT1), taurine transporter (TauT) and GABA transporter 2 (GAT2) were known to be responsible in cellular ALA uptake. Inhibitors of highly expressed transporter(s) in normal cell but not in cancer cells would serve as a new drug to increase specificity of ALA-PDT. The role of transporters is also believed to be highly influential on the efficiency of ALA-PDT under different cell density.

Seven human cell lines, composed of normal and cancerous cell lines were used in this study. Expression levels of four transporters in these cell lines were evaluated using Western blotting. Transporters inhibition studies was carried out via the co-addition 1 mM ALA and ibuprofen (PEPT1 inhibitors) or tryptophan (PAT1 inhibitors) into respective dishes. The samples were incubated for 24 hours and the levels of intracellular and extracellular PpIX are measured using high performance liquid chromatography (HPLC). For cell viability assay, DU145 cells were first seeded for 24 h and incubated with 1 mM ALA in culture media under 5% CO2 gas at 37 ºC in the dark for 4 h. The samples were then exposed to light irradiation for 5 min before being incubated again in the dark under 5% CO_2 at 37 ºC for 24 h. The number of living cells were quantified using trypan blue & Countess II FL cell counter.

The expression levels of transporters involved in ALA uptake in various normal / cancer cell lines were studied. No correlation was observed between the expression levels of transporters among cell lines of same origin. Inhibition of highly expressed transporters resulted in a significant reduction of PpIX levels, possibly due to decrease in ALA uptake. Two major findings were obtained from these studies, PEPT1 being expressed only in normal lung cells but not in its cancerous counterpart; and PAT1, which was expressed only in normal prostate cells but not in its cancerous counterpart. The inhibition of these transporters showed a significant decrease in PpIX production only in normal cells but not in cancer cells. These results suggest ibuprofen and tryptophan might be useful in increasing its specificity towards tumours in lungs and prostate cells. The above results showed that usage of drugs, ibuprofen and tryptophan, targeted specifically to highly expressed transporters in normal cells is essential in reducing PpIX accumulation in normal cells. This is important in increasing the specificity of ALA-PDT and ALA-PDD in tumours.

Next, the efficiency of ALA-PDT under different cell density were being studied using trypan blue solution. Samples of high cell density showed a sharp decrease in cell viability of DU145 cells to only about one-fifth of the control following light irradiation, while no

significant difference in cell viability was observed in the low cell density samples. This result suggests that the cancer-killing effect of ALA-PDT appears to be more potent in cancer of higher cell density. Next, it is observed that the expression levels of ALA uptake transporters gradually increase as the cell density increase.

Previous studies have showed the importance of PpIX level in determining the effectiveness of ALA-PDT. Therefore, the concentration of PpIX in DU145 cells under different cell density was studied. It is observed that PpIX level increases following the increase in cell density. This finding coincided with previous findings whereby ALA-PDT was observed to be more effective at high cell density condition. This study also showed that inhibition of Yes-associated protein (YAP), an oncogene involved in cell density and cell contact inhibition, using verteporfin, resulted in decrease in protein expression levels of malignancy markers and ALA uptake transporters, suggesting a possible regulatory role for these proteins. In a nutshell, these data suggest that ALA uptake increase due to higher expression of transporters at higher cell density, which leads to an increase in efficiency of ALA-PDT at high density. Since the expression level of malignancy markers increase as cell density increase, there is also a potential connection whereby ALA-PDT may be more effective in cancer cells of higher malignancy. This study also suggests possible role of YAP in regulating the expression levels of malignancy markers and ALA uptake transporters.

(796 words)