46P Enhanced 5-aminolevulinic acid (ALA)-based Photodynamic Therapy (PDT) Efficacy by hormones in uterine sarcoma cells via upregulation of proporphyrinogen oxidase

Chu ESM[1*], Wu RWK[1], HY Lau[1], Huang Z[2]

[1]School of Medical and Health Sciences, Tung Wah College, HKSAR (email: elliechu@twc.edu.hk)

[2]MOE Key Laboratory of Photonics Science and Technology for Medicine, Fujian Normal University, Fuzhou, China

INTRODUCTION

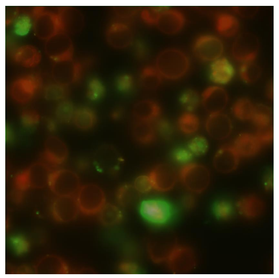
Photodynamic Therapy (PDT) is an FDA approved cancer treatment modality for various cancers. It induces a photochemical reaction with exogenous photosensitizer (PS) in cancer cells upon light irradiation with oxygen, which generates reactive oxygen species (ROS) leading to cancer cell death. Studies showed that hormones enhanced accumulation of photosensitive protoporphyrin IX (PpIX) generated by ALA PS in human endometrial cells, implying the increased PpIX potentially boost up ALA-based PDT effect in hormonal dependent cancers. Yet the underlying mechanism remains unexplored.

METHODS

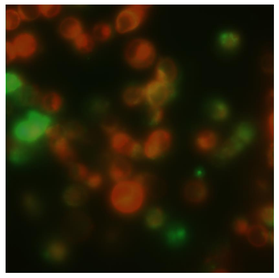
Uterine sarcoma cells were cultured with fluctuated 17β-estradiol (E2) and progesterone (P) levels as human menstrual cycle, then treated with exogenous Hexyl-ALA compared with normal cultured cells. PpIX localization and accumulation in the cells were determined by confocal microscopy and flow cytometry respectively. Hexyl-ALA-PDT effect was evaluated by MTT assay with sulfentrazone (Sul), an inhibitor of proporphyrinogen oxidase (PPOX) - a limiting factor of PpIX in heme pathway. The PPOX expression in cells was quantified by flow cytometry.

RESULTS

Localisation of PpIX in Mitochondria

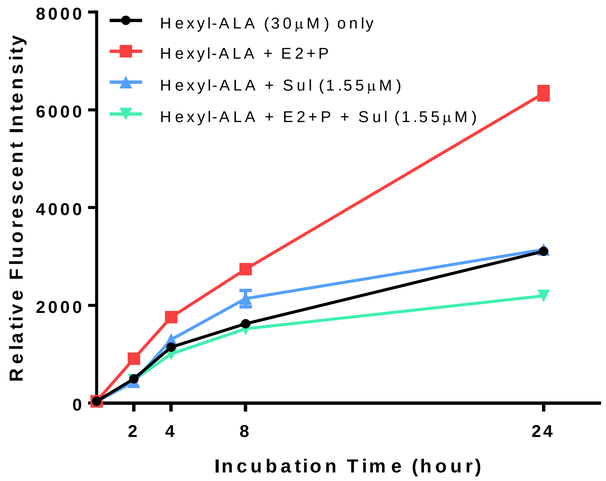


Co-localization (yellow) of overlay images of Hexyl-ALA (red) with MitoTracker (green) (1000x)



Co-localization (yellow) of overlay images of Hexyl-ALA with E2+P (red) with MitoTracker (green) (1000x)

Generation of PpIX by Hexyl-ALA

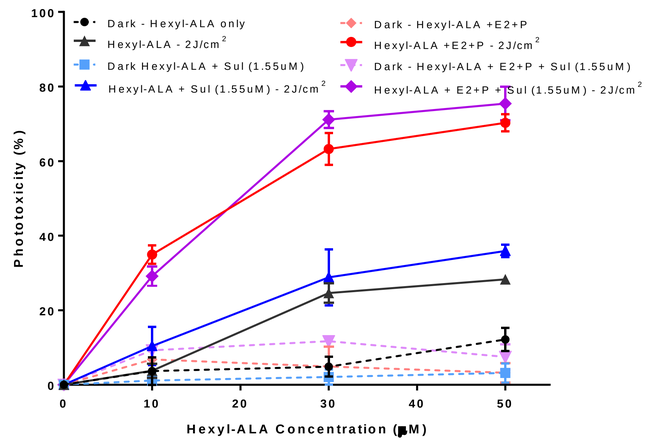


The generation and accumulation of PpIX by Hexyl-ALA in uterine sarcoma was highly enhanced in the presence of E2 and P in time-dependent manner.

The presence of the PPOX inhibitor, sulfentrazone (Sul), inhibited the generation and accumulation of PpIX.

RESULTS

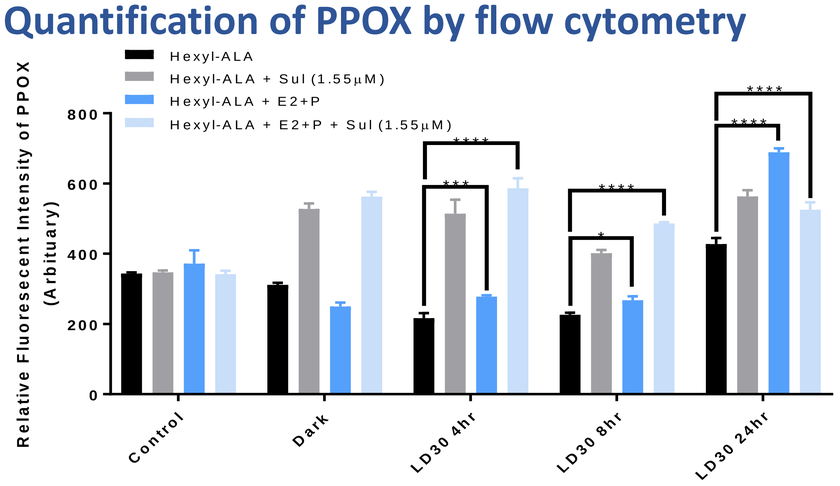
Phototoxicity of Hexyl-ALA-PDT in uterine sarcoma cells



At 4hr, the phototoxicity increased from 20% to 60% at Hexyl-ALA (30 μM, 2J/cm^2) compared with hormones respectively.

Addition of Sul, the inhibitor of PPOX, further enhanced the phototoxicity to 70% indicating PPOX might involved in the phototoxic pathway of Hexyl-ALA-PDT.

Quantification of PPOX by flow cytometry



At LD_{30} of Hexyl-ALA-PDT, the presence of both hormones E2 and P significantly induced the PPOX expression in a time-dependent manner compared with Hexyl-ALA-PDT alone.

The presence of PPOX inhibitor, Sul, further triggered PPOX expression especially in the presence of hormones.

PPOX level enhanced by hormones implying the hormonal micro-environment boost up Hexyl-ALA-PDT effect in uterine sarcoma cells via up-regulation of PPOX through heme pathway.

CONCLUSION

This study showed that hormones played a vital role in enhancing Hexyl-ALA PDT effect via up-regulation of PPOX.

The simulated hormonal microenvironment culture model is suitable to study Hexyl-ALA-PDT effect in hormonal dependent cancers.

DECLARATION OF INTERESTS

The authors declared that there is no financial interests to be disclosed.

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