Potential therapeutic efficacy of photodynamic therapy on triple negative breast cancer in hormonal microenvironment

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hours, followed by 3J light activation. The changes in spheroids' size and morphological characteristics at post-PDT 24 hours and 72 hours.

4. Results

The spheroid size increased from 393.7μm±15.7μm to 944.6μm±81.4μm with the initial cell densities increased from 5,000 to 60,000 cells/well at day 3 culture. Spheroids formed by 5,000-20,000 cells were less varied in size and with smooth round edges, while spheroids formed by 30,000 and 60,000 cells were observed with irregular edges and a dense core.

The PpIX accumulation was spheroid size dependent, with an increase of PpIX accumulation in larger spheroids. Interestingly, PpIX accumulation started from the peripheral regions to the core in small spheroids, with maximum PpIX accumulation at the core at 26 hours of incubation. However, the PpIX accumulation in large spheroids mainly remained in the peripheral regions. This finding could be explained by the densities of spheroids and the diffusion distance as 5-ALA are more difficult to diffuse into the core of the spheroids when the spheroid size and volume increase.

1mM 5-ALA-PDT with 3J light activation showed an inhibitory effect on spheroids formed by 5,000 and 30,000 cells. For post-PDT 24 to 72 hours, the periphery size of control spheroids formed by 5,000 and 30,000 cells increased from 83.9μm±25.3μm to 103.1μm±33.9μm and from 111.0μm±32.2μm to 131.1μm±42.3μm, respectively. However, the periphery size was inhibited by 5-ALA-PDT on PDT-treated spheroids formed by 5,000 and 30,000 cells, with a decrease from 84.5μm±26.2μm to 59.0μm±23.7μm and from 99.8μm±45.3μm to 97.0μm±46.6μm, respectively. Loose structures with cells disaggregated from the spheroids were also observed in PDT-treated spheroids.

5. Conclusion

The NPC/CNE2 spheroids with different sizes can be formed by different initial cell densities. The size of spheroids affects the PpIX accumulation and PDT efficacy. More in-depth studies are deserved for the optimization of NPC/CNE2 3D spheroid formation for PDT studies.

Disclosures if required

The authors confirmed that there are no conflicts of interest should be declared.

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Potential therapeutic efficacy of Photodynamic Therapy on triple negative breast cancer in hormonal microenvironment

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Significance: There are seldom studies on the determination of cancer treatment efficacy related to normal hormonal tumor microenvironment on triple negative breast cancer (TNBC). This study aimed to determine Hexyl-ALA-PDT efficacy on TNBC in a simulated hormonal microenvironment.

Approach: The 3D spheroids of TNBC cells (MDA-MB-231) were generated in the hormonal supplemented microenvironment. The accumulation of PpIX, phototoxicity, the ROS level and p-ULK1 expression level mediated by Hexyl-ALA-PDT were determined by confocal microscopy, MTT assay and flow cytometry.

Results: A lower Hexyl-ALA concentration was required to achieve the same lethal dose of LD50 with hormonal supplement in 3D spheroids. It was found that the ROS level was increased at lower Hexyl-ALA-PDT dose in hormonal microenvironment, which also correlated the decrease of p-ULK1 expression by PDT.

Conclusions: Hexyl-ALA-PDT increased ROS level in TNBC in hormonal microenvironment; and with the reduced p-ULK1 expression thus indicating autophagy of cell death might be triggered.

Keywords: Photodynamic Therapy, Triple Negative Breast Cancer, Hormonal microenvironment, 3D spheroids, Hexyl-ALA

1. Introduction and Background

Cancer cell growth can be facilitated by hormones normally present in the physiological microenvironment [1,2,3]. Studies usually focused on cancer treatment effect with only a single dose of hormone, such results over-estimated the treatment efficacy and ignored the hormonal effect on the treatment efficacy in normal physiological tumor microenvironment [4, 5]. Our pilot studies evidenced the hormonal enhancement of photodynamic therapy (PDT) efficacy in hormonal dependent cancers

[6,7]. However, such hormonal effects in hormonal-independent cancers are usually ignored by the researchers, especially in most *in vitro* culture cancer models.

2. Aims

This study aims to elucidate the hormonal effect on hexyl-aminolaevulinic acid (Hexyl-ALA)-PDT efficacy in triple negative breast cancer (TNBC) 3D culture.

3. Methods

The 3D spheroids of MDA-MB-231 cells were formed by the liquid overlay agarose-based technique in hormonal supplemented microenvironment with estrogen (E2), progesterone (P), or estrogen and progesterone (E2+P) [8]. The intracellular distribution of protoporphyrin IX (PpIX) generated from Hexyl-ALA in the spheroids was detected by confocal microscopy and flow cytometry. Phototoxicity was determined by MTT assay. Hexyl-ALA-PDT induced hypoxia was determined via the distribution of reactive oxygen species (ROS) level by confocal microscope and flow cytometry. The mode of cell death was determined by the autophagy-related phosphorylated-unc-51 like kinase 1 (p-ULK1) expression by flow cytometry.

4. Results

Hexyl-ALA-PDT was effective to MDA-MB-231 3D spheroids in hormonal microenvironment. Comparing to Hexyl-ALA-PDT (50μM, 4J/cm^2), a lower H-ALA concentration (30μM, 4J/cm^2) supplemented with E2 and P achieved the same lethal dose of 50 (LD50). An increase in ROS level was observed immediately at post-PDT in hormonal microenvironment, which correlated with the reduced p-ULK1 expression. However, only 10% of ROS decreased with E2+P at 4hr post-PDT; whereas other conditions with single hormone and without hormone lead to more than 30% decrease of ROS indicating the hormonal condition might preserve the spheroids from hypoxia [9].

5. Conclusion

Hormones modulated the production of ROS by Hexyl-ALA-PDT and reduced p-ULK1 expression in 3D MDA-MB-231 spheroids indicating hypoxia and autophagy might be enhanced. Further mechanistic elucidation on hormonal modulated PDT in TNBC through p-ULK1 induced autophagy and enhanced ROS production level remains to be explored.

Disclosures if required

The authors confirmed that there are no conflicts of interest should be declared.

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NIR dye-based electrospun fibers for antimicrobial photodynamic therapy

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Abstract: Antimicrobial photodynamic therapy (aPDT) has emerged as a promising strategy to improve antimicrobial treatment and face the antibiotic resistance to conventional therapy. Among the different photosensitizers, Near Infrared (NIR) polymethine dyes (Squaraines, SQs and Cyanines, CYs) have attracted considerable attention although, their poor aqueous solubility and stability still limit their application. Their incorporation inside nanoparticles (NPs) or into electrospun fibers, could help to prevent dye aggregation, protect their photochemical properties, and play a key role in the infection treatment.
Cys and SQs were characterized before and after the incorporation, showing improved optical properties and photostability. In addition, their ability to produce Reactive Oxygen Species (ROS), responsible of bacterial death, was evaluated, proving that the ROS generation ability is preserved after the incorporation. Furthermore, *in vitro* antimicrobial studies against Gram-negative and Gram-positive models showed a good antimicrobial effect after the irradiation, confirming the potential of these nanophotosensitizers for aPDT in the local treatment of infections.

Keywords: Antimicrobial photodynamic therapy, Near Infrared (NIR) polymethine dyes, Squaraines and Cyanines, electrospun fibers

1. Introduction and Background

A possible strategy to fight the antimicrobial resistances in acute wounds is the application of antimicrobial photodynamic therapy (aPDT) [1].