Photodiagnosis and Photodynamic Therapy 28 (2019) 159–165

Contents lists available at ScienceDirect

Photodiagnosis and Photodynamic Therapy

journal homepage: www.elsevier.com/locate/pdpdt

Photodynamic therapy enhances skin cancer chemotherapy effects through autophagy regulation

Lili Zhang[1], Zhoujing Ji[1], Jie Zhang*, Shengju Yang*

Department of Dermatology, Affiliated Hospital of Nantong University, Nantong, 226001, PR China

ARTICLE INFO

Keywords:
Photodynamic therapy
Autophagy
Skin cancer
Apoptosis
Chemotherapy

ABSTRACT

Non-melanotic cutaneous cancers and melanoma are the main common type skin cancers worldwide. Despite many therapeutic options, therapeutic efficacy is not satisfied in all patients with advanced skin cancers, especially in melanoma. Photodynamic therapy (PDT) is a technology for skin disease treatment because of its high effectiveness, has no drug resistance and is easy to use compared with traditional therapy. Our previous study explored that autophagy plays an important role in the formation and development of SCC. But there was no evidence about the association between PDT with autophagy in skin cancers and the mechanism is also still unclear. In the study, we want to explore the effects of 5-aminolevulinic acid-PDT (ALA-PDT) on the skin cancers through autophagy regulation. The result showed that autophagy was regulated by ALA-PDT combined with or without 3-Methyladenine (3-MA) or 5-Fluoracil (5-FU), the proliferation of skin cancer cells A431 and A375 were suppressed while the apoptosis were induced by ALA-PDT and the effects can be enhanced by 3-MA or 5-FU pretreated. The results suggest that autophagy regulation may be a key point of ALA-PDT therapy; ALA-PDT combined with the chemotherapy of 3-FU or 5-FU may be a new strategy for treatment of skin cancers including non-melanotic cutaneous cancers or melanoma.

1. Introduction

Non-melanotic cutaneous cancers and melanoma are two types of human skin neoplasms, and non-melanotic cutaneous cancers are the most common skin cancers worldwide and include squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) [1]. SCC originate in the skin or adnexal cutin cell formation, is invasive and is a malignant skin cancer, accounting for 15% of malignant epithelial tumors, which also accounts for 20% of non-melanotic skin cancer [2]. Moreover, melanoma is the most serious and highly aggressive form of skin cancer [3]. The pathogenesis of skin cancers may be related to ultraviolet irradiation, exposure to certain chemicals, precancerous skin diseases, trauma, scars and immunosuppressive agents' application, but the mechanism is still unclear. Most of SCC originated from skin in the head and neck region, which was caused by years of chronic ultraviolet radiation, mainly caused by outdoor work [4,5]. In most cases, SCC can be cured easily by simple resection or radiation therapy, but locally advanced tumors can also present local recurrence, lymph nodes or distant metastasis [6,7]. There are numerous therapeutic options for melanoma such as chemotherapy, immunotherapy and gene therapy, but advanced melanoma prognosis remains severe [8]. Thus, the study of the pathogenesis, treatment and prevention of recurrence and metastasis of skin cancers is a key problem that needs to be solved in the field of dermatology.

Photodynamic therapy (PDT) is a technology based on photodynamic effect for disease treatment [9,10]. PDT is a popular dermatological treatment involving light and a photosensitizer used in conjunction with molecular oxygen to elicit cell damage and even death, further leading the damage of diseased tissues [11,12]. PDT has become a effective strategy for skin cancer treatment because of its advantages, i.e. small trauma, optional treatment area, high effectiveness, no drug resistance or toxic side effect; used completely independently or in combination with other therapies; non-invasive, compared with traditional therapy [13,14]. Autophagy is an intracellular degradation pathway involving the degradation of cytoplasmic components in all cells. Recent studies showed that autophagy played an important role in the formation and development of tumors [15]. Under normal physiological conditions, cell autophagy is conducive to cell self-stabilization. When a stress response is triggered, cell autophagy can limit the chromosomal instability, thereby reducing the accumulation of cancer-causing mutations; also limit oxidative stress and reduce intratumoral necrosis and inflammation to prevent the accumulation of toxic or

* Corresponding authors at: Affiliated Hospital of Nantong University, 20 Xisi Road, Nantong 226001, PR China.
E-mail addresses: zhangjie7401@163.com (J. Zhang), yangshengju80@163.com (S. Yang).
[1] These authors contributed equally to this paper.

https://doi.org/10.1016/j.pdpdt.2019.08.023
Received 8 April 2019; Received in revised form 19 August 2019; Accepted 20 August 2019
Available online 21 August 2019
1572-1000/

Table 1
Sequences of RT-qPCR primers.

Gene Name	Sequence (5'-3')	
LC3	F	GAGCTCCAAGTGAGCACATTC
	R	ACTGCTGGAATTACAAGTTCCTAT
BAX	F	TGACGGCAACTTCAACTG
	R	CGGAGGAAGTCCAATGTC
Bcl2	F	TGTGTGGAGAGCGTCAAC
	R	CCAGCCTCCGTTATCCTG
Survivin	F	ACCGCATCTCTACATTCAAG
	R	CAAGTCTGGCTCGTTCTC
β-actin	F	TTGCCGACAGGATGCAGAAGGA
	R	AGGTGGACAGCGAGGCCAGGAT

F, Forward primer; R, Reverse primer.

carcinogenic damaged proteins and organelles, and thereby inhibit cell carcinogenesis [16,17].

In our previous study, we explored the role of autophagy in SCC and was associated with the 3-MA or 5-FU based chemotherapy sensitivity [18]. In this study, the effects of 5-aminolevulinic acid-PDT (ALA-PDT) on the skin cancers and combination with 3-MA or 5-FU through the autophagy were investigated, and further to clarify the mechanism of ALA-PDT enhanced chemotherapy effects in skin cancer cells.

2. Materials and methods

2.1. Cells and culture

Human epidermal squamous carcinoma cell line A431 and melanoma cell line A375 were obtained from Biomics Biotechnologies Co Ltd (Nantong, China), both cells were cultured in high-glucose Dulbecco's modified Eagle's medium (DMEM) and supplemented with 10% fetal bovine serum (FBS) (Thermo Fisher Scientific, USA) at 37 °C in a cell culture incubator with 5% CO_2.

2.2. Chemicals and PDT treatment

5-Aminolevulinic acid (ALA) (Sigma-aldrich, USA) was dissolved in 0.9% sodium chloride to form a 40 mM solution, and was kept at 4 °C before use. A light-emitting diode (LED) phototherapy apparatus (Wuhan Yage Optic and Electronic technology Co., Ltd, China) with peak emission at the wavelength of 633 nm ± 10 nm was used for as the light source for PDT, and different irradiances can be simultaneously available for comparative PDT studies by using different optical attenuators. Cells were treated with 5-Fluoracil (5-FU) and 3-Methyladenine (3-MA) at concentration of 10 μg/ml and 4 mM respectively for 3 h and then incubated with 5-ALA (2 mM) for 3 h prior to light irradiation. The condition of irradiance at the surface of culture plate was 20 mW/cm^2 for 10 min and cells were continue cultured for 24 h.

2.3. MTT assay

The proliferation of cells was detected by MTT assay. Briefly, A431 and A375 cells were plated into 96-well plates and grown for 24 h at 5×10^4 cells/ml before treatment. The cells were washed three times in PBS buffer (pH7.4) when treated as above at 0, 24, 48 or 72 h, and incubated in 100 μl/well of PBS and 10 μl MTT working solution (Promega, USA) at 37 °C for 30 min. Finally, the OD values of the cells were measured by fluorescence microplate reader (Bio-Rad, USA) at 570 nm wavelength.

2.4. Hoechst fluorescent staining

The apoptosis morphological changes of cell nucleus induced were detected by Hoechst fluorescent staining. In brief, 5×10^4 cells were seeded into 24-well plates with a small round glass coverslip in each well and cultured in a 37 °C humidified incubator overnight. After been treated as above, the media was removed and discard first and washed

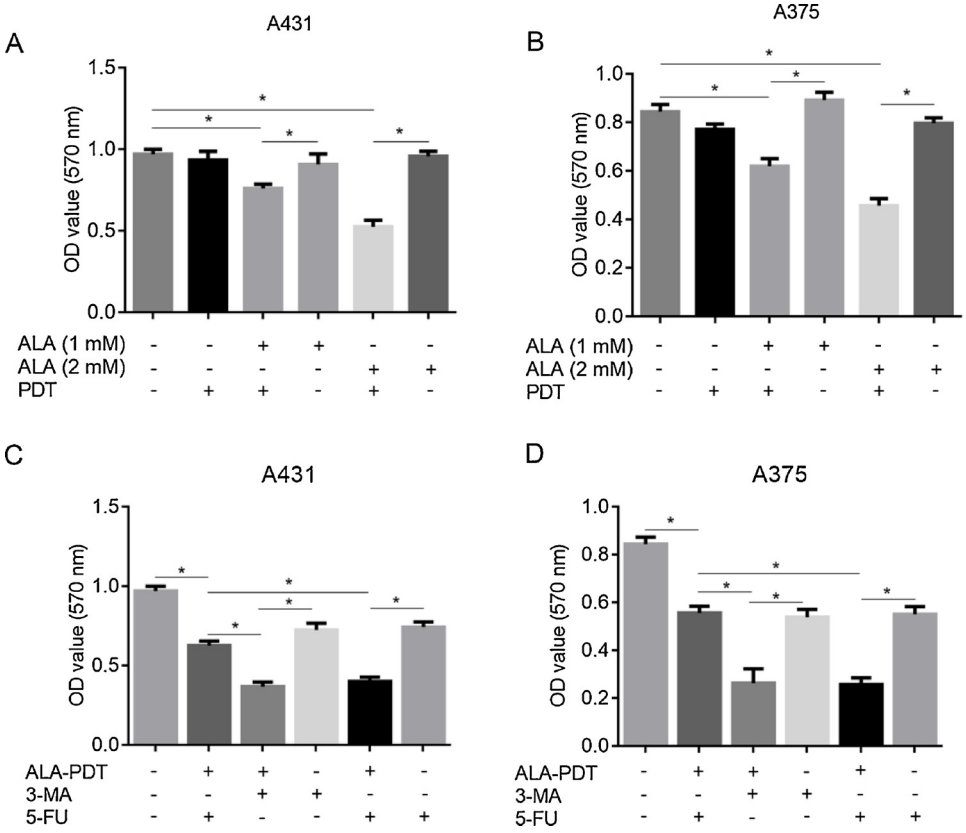

Fig. 1. Effects of ALA-PDT on the proliferation of A431 and A375 cells. A. The proliferation of A431 cells inhibited by ALA-PDT; B. The proliferation of A375 cells was inhibited by ALA-PDT; C. The proliferation of A431 cells was inhibited by combination of ALA-PDT with 3-MA or 5-FU; D. The proliferation of A375 cells was inhibited by combination of ALA-PDT with 3-MA or 5-FU. *$P < 0.05$, compared with untreated cells.

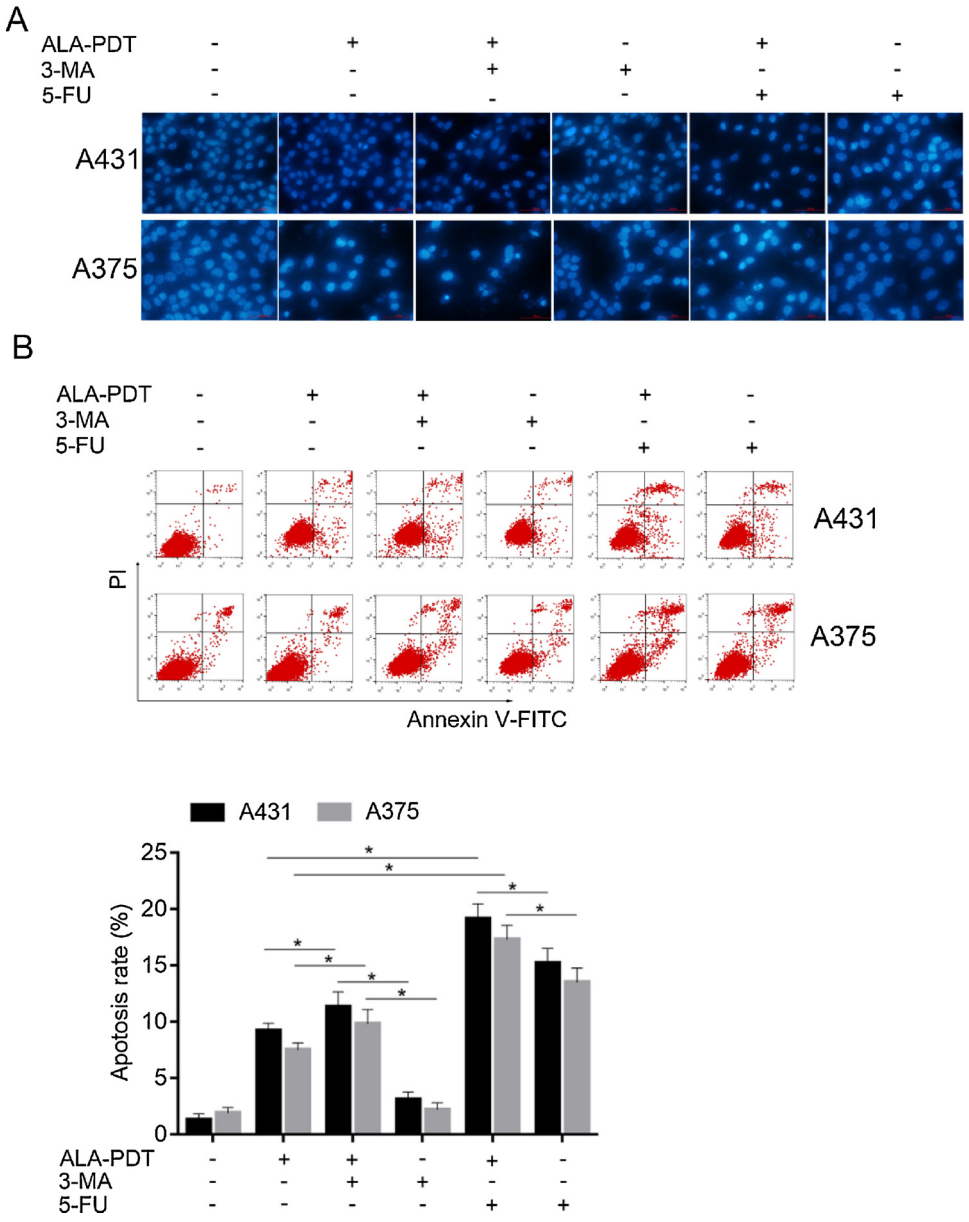

Fig. 2. The apoptosis of A431 and A375 cells induced by ALA-PDT combined with or without 3-MA or 5-FU. A. Apoptotic cells were detected by Hoechst staining. The scale bar indicates 50 μm. B. The apoptosis rate of cells was detected by FCM. *$P < 0.05$, compared with untreated cells.

by PBS, the cells were fixed with 4% formaldehyde solution (Sangon Biotech, China) for 30 min at room temperature, then washed three times with PBS for 5 min each, Hoechst 33258 (10 mg/ml) (1:1000 diluted in PBS) was used for cell nuclei staining for 30 min. After been washed in PBS, the cells were mounted in antifade mounting medium. The stained cells were observed under an immunofluorescence microscopy protect from light.

2.5. Flow cytometry assay

Flow cytometry after Annexin V-FITC/PI staining with Annexin V-FITC Apoptosis Detection kit (Sigma-aldrich, USA) was used to evaluate the rate of cell apoptosis. Briefly, cells were seeded onto 6-well plates and growth for 24 h. Post 48 h treatment as above, the media was removed and discard first and washed by PBS, and then the confluent cells were dissociated with 0.25% trypsion-0.02% ethylenediaminetetraacetic acid (EDTA) (Thermo Fisher Scientific, USA), and 1×10^5 cells were re-suspended in PBS, after been centrifuged at 1000 rpm for 5 min, the cells were re-suspended in 195 μl Annexin V-FITC binding buffer, then added 5 μl Annexin V-FITC and incubated for 10 min at room temperature. After been centrifuged at 1000 rpm for 5 min, the cells were re-suspended in 10 μl Propidium Iodide (PI) for 15 min on ice protect from light. At last, the cells were detected using flow cytometry (BD Biosciences, USA).

2.6. Real-time quantitative PCR (RT-qPCR)

The mRNA expression levels of relative genes were detected by RT-qPCR method and relative mRNA levels were analyzed according to $2^{-\Delta\Delta Ct}$ method [19]. In brief, 5×10^3 cells were seeded onto 96-well plates and growth for 24 h before treatment. Post 48 h treatments as above, total RNA of cells was extracted using TRIzol® reagent (Thermo Fisher Scientific, USA) according to the manufacturers' instructions. RT-qPCR reactions were carried out using the SuperScript™ III Platinum™ SYBR™ Green One-Step qRT-PCR kit (Thermo Fisher Scientific, USA) according to the manufacturer's manual, and the house-keeping gene β-actin was used as an internal control. The used primers were obtained from Biomics Biotechnologies Co Ltd (Nantong, China) and sequences

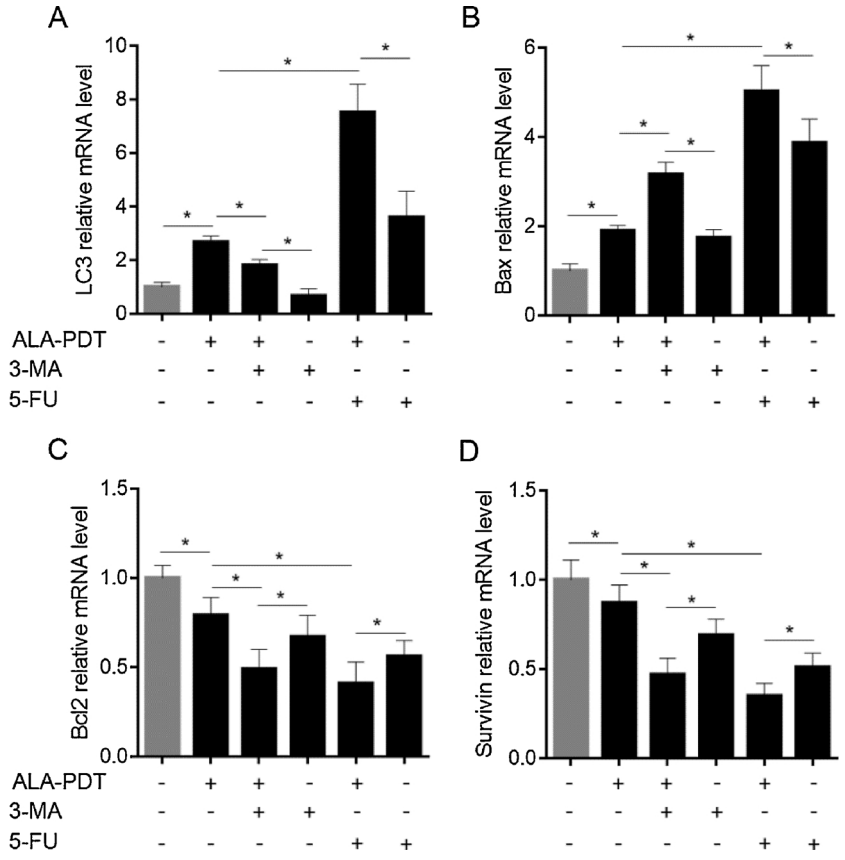

Fig. 3. The expression levels of LC3 (A), Bax (B), Bcl2 (C) and Survivin (D) affected by ALA-PDT combined with or without 3-MA or 5-FU treatment detected by RT-qPCR in A431 cells. *$P < 0.05$, compared with untreated cells.

were shown Table 1.

2.7. Western blot

The protein expression levels of relative genes were detected by Western blot assay. Briefly, a total of 1×10^6 cells were seeded onto 6-well plates and growth for 24 h before treatment. Post 48 h treatments as above, the total proteins of cells were extracted using cell RIPA lysis and extraction buffer (Thermo Fisher Scientific, USA) on ice. The proteins were separated by SDS-PAGE and transferred onto PVDF membranes (Merckmillipore, USA), and then, the membrane was incubated with primary antibody, a rabbit anti-human LC3, Bax, Bcl2, Survivin, Caspase 3, Caspase 8 or Caspase 9 antibody (1:500 dilution) respectively, a mouse anti-human β-actin antibody (1:1000 dilution) used as internal control (all antibodies were from Abcam, USA). After been washed with TBST, the membrane was incubated with secondary antibody, a horseradish peroxidase (HRP)-conjugated IgG (1:2000 dilution) (Abcam, USA) for 2 h at room temperature. At last, the specific protein was detected with Pierce ECL substrate (Thermo Fisher Scientific, USA).

2.8. Monodansylcadaverine (MDC) fluorescent staining

The procedure of cell culture and treatments was same as above Hoechst fluorescent staining. Briefly, post 24 h treatments, the cells were washed by PBS for 5 min twice, 0.05 mmol/l MDC (Sigma-Aldrich, USA) was added to each coverslip with cells, and incubated for 1 h at 37 °C protect from light, and then washed with PBS, the coverslips were fixed with 4% formaldehyde solution (Sangon Biotech, China) for 15 min and mounted. At last, the cells were observed under an immunofluorescence microscopy protect from light.

2.9. Transmission electron microscopy

The method was performed according to our previous study [18]. Cells were plated and treated as described above. Post 48 h treatments, the cells were collected and fixed with ice-cold 2.5% glutaraldehyde in PBS for 2 h at 4 °C. After been washed in PBS, the cells were fixed in 1% OsO4, and dehydrated through a graded series of ethanol (50–90%) for 20 min. After been embedded and fixed, the cells were cut into 70 nm sections. 3% uranyl acetate and lead citrate staining was used for examination using HT7700 transmission electron microscope (HITACHI, Japan).

2.10. Statistical analysis

In this study, all experiments were performed three times independently and the data are shown as mean values ± standard deviation (SD). Statistical analysis was performed by SPSS19.0 software. Comparisons between two groups were performed by Student's t-test. Comparisons among multiple groups were performed by one-way analysis of variance (ANOVA) followed by a Dunnett post hoc test. All *P*-values are based on a two-tailed statistical analysis. A *P* value less than 0.05 was considered as statistical significance.

3. Results

3.1. Inhibition effects of ALA-PDT on the proliferation of A431 and A375 cells

MTT assay was used to detect the cell proliferation. Both A431 and A375 cells were pre-treated with ALA at the concentration of 1 mM or 2 mM for 3 h and then treated with PDT, the result showed that the proliferation of A431 or A375 cells were inhibited by the treatment of

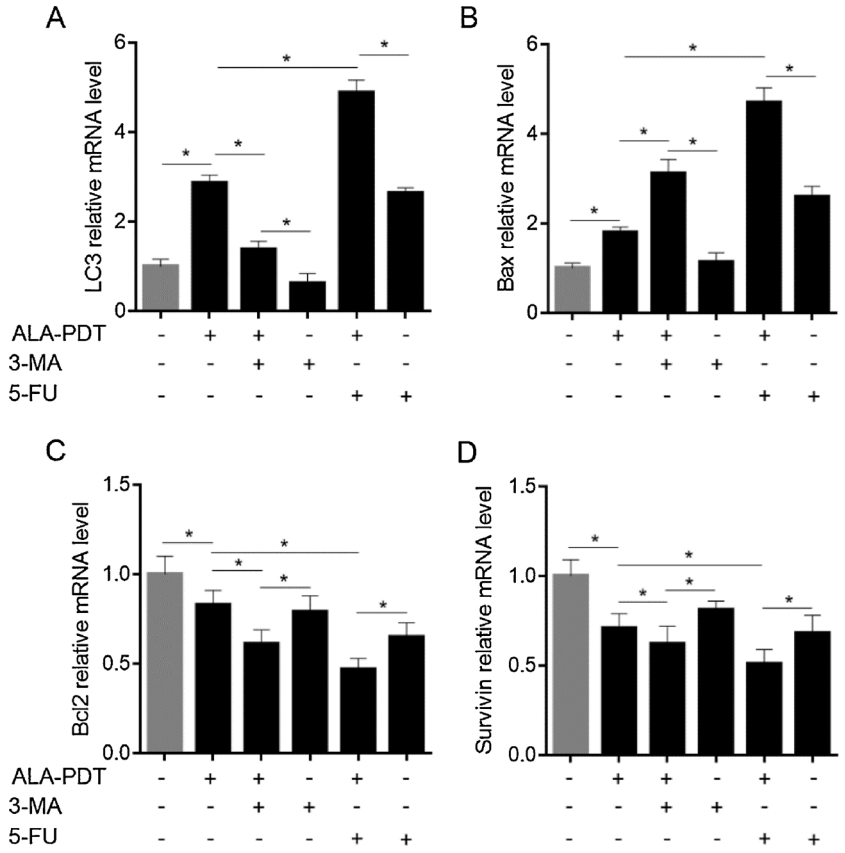

Fig. 4. The expression levels of LC3 (A), Bax (B), Bcl2 (C) and Survivin (D) affected by ALA-PDT combined with or without 3-MA or 5-FU treatment were detected by RT-qPCR in A375 cells. *$P < 0.05$, compared with untreated cells.

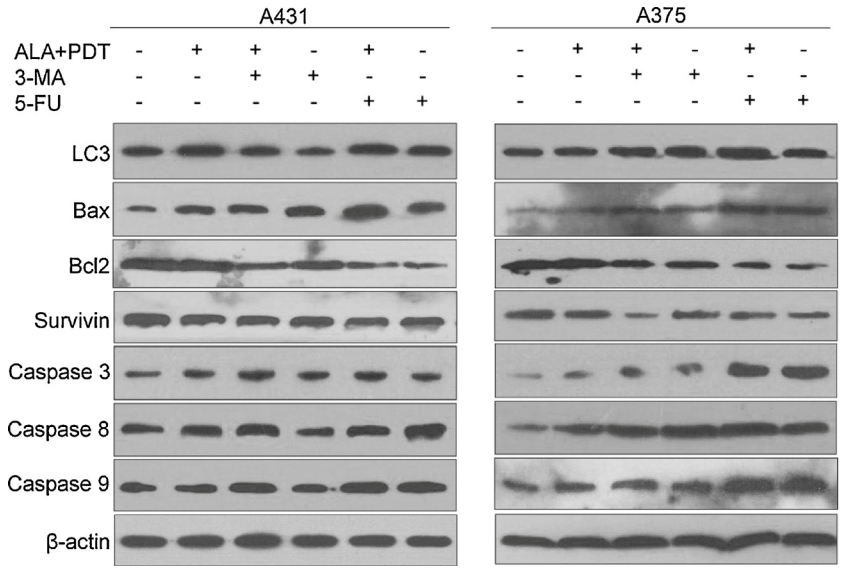

Fig. 5. The expression levels of LC3, Bax, Bcl2, Survivin, activated Caspase 3, Caspase 8, and Caspase 9 affected by ALA-PDT combined with or without 3-MA or 5-FU treatment were detected by Western blot. *$P < 0.05$, compared with untreated cells.

ALA-PDT both at the concentration of 1 mM or 2 mM significantly, especially 2 mM ALA treated cells ($P < 0.05$), compared with untreated cells or only PDT treated cells (Fig. 1A and B).

3.2. ALA-PDT combined with 3-MA or 5-FU inhibited the proliferation of A431 and A375 cells

Both A431 and A375 cells were treated with 10 μg/ml 5-FU or 4 mM 3-MA respectively for 3 h and then treated with ALA + PDT using 2 mM ALA, and the cell proliferation was detected by MTT assay at indicated time points. The results showed that the proliferation of A431 and A375 cells were both inhibited by ALA-PDT with 3-MA or 5-FU, especially treated with 5-FU ($P < 0.05$), compared with untreated cells or only PDT treated cells (Fig. 1C and D).

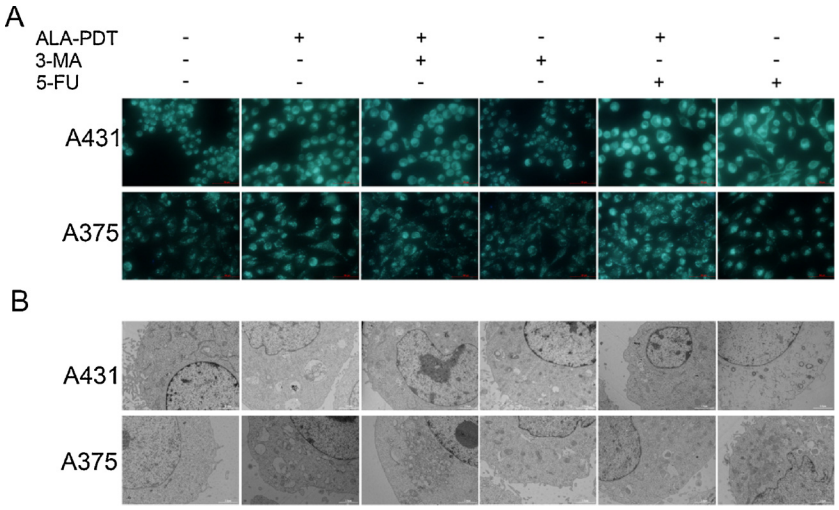

Fig. 6. Autophagy regulated by ALA-PDT combined with 3-MA or 5-FU in A431 and A375 cells. A. Morphology of autophagosomes in A431 and A375 cells was detected by MDC staining assay. The scale bar indicates 50 μm. B. Cell morphology observed by transmission electron microscopy. The scale bar indicates 2.0 μm.

3.3. ALA-PDT combined with 3-MA or 5-FU promoted the apoptosis of A431 and A375 cells

The morphology change of cells was detected by Hoechst fluorescent staining of cell nucleus and observed with microscope. To compare with untreated cells, the apoptosis of A431 or A375 cells was induced obviously as a result of the dead cell numbers of A431 or A375 increased with the treatment of ALA-PDT combined with or without 3-MA or 5-FU (Fig. 2A). Annexin-V-FITC/PI double staining with flow cytometry was also used for cell apoptosis detection, the result showed that the apoptosis rate of A431 and A375 increased significantly with the treatment of ALA-PDT combined with or without 3-MA or 5-FU ($P < 0.05$), compared with untreated cells (Fig. 2B).

Moreover, the apoptotic genes Bax, Bcl2 and survival gene Survivin were used to monitor the cell apoptosis occurrence. The mRNA levels detected by RT-qPCR showed that Bax increased while Bcl2 and Survivin decreased significantly with the treatment of ALA-PDT combined with or without 3-MA or 5-FU ($P < 0.05$) in A431 and A375 cells, compared with untreated cells (Figs. 3 and 4). The protein levels detected by Western blot showed that Bax increased while Bcl2 and Survivin decreased significantly in A431 and A375 cells, also the apoptotic genes Caspase 3, 8 and 9 were activated with the treatment of ALA-PDT combined with or without 3-MA or 5-FU ($P < 0.05$), compared with untreated cells.

3.4. Autophagy regulated by ALA-PDT combined with 3-MA or 5-FU in A431 and A375 cells

Autophagy triggered in A431 and A375 cells was monitored by detection of LC3 expression, and the result showed that LC3 mRNA and protein levels increased in A431 and A375 cells treated by ALA-PDT combined with or without 3-MA or 5-FU, while LC3 mRNA and protein levels decreased in 3-MA treated cells (Fig. 3A and Fig. 5). MDC staining assay was used to detect the formation of autophagy. To compare with untreated cells, the number of MDC stained cells increased obviously in A431 and A375 treated by ALA-PDT combined with or without 3-MA or 5-FU, while decreased in 3-MA treated cells (Fig. 6A).

Transmission electron microscopy showed that the autophagic vacuoles were formed obviously in the cytoplasm of A431 and A375 cells treated by ALA-PDT combined with or without 3-MA or 5-FU, but there was no evident autophagic vacuoles formed in 3-MA treated cells, compared with untreated cells (Fig. 6B).

4. Discussion

With the development of photodynamic research, photosensitizer and light source, PDT has been widely used in tumor treatment, especially for skin diseases and tumors with a wide range of prospects, and has been successfully used in the treatment of skin tumors such as precancerous skin cancer, SCC in situ and basal cell carcinoma [20,21]. In the study, we found that the proliferation of skin cancer cells A431 and A375 were both inhibited by ALA-PDT with 1 mM or 2 mM ALA. Furthermore, the proliferation of A431 and A375 cells were also inhibited by 3-MA or 5-FU, when combined with ALA-PDT, the inhibition effects were enhanced.

Autophagy mediated tumor inhibition can remove damaged oxidized organelles, thereby preventing genomic instability due to accumulation of toxic oxygen free radicals [22]. Katheder NS et al. found that surrounding cells degrade their own proteins through the process of autophagy, and then release amino acids for the absorption and use of cancer cells [23]. Autophagy microtubule associated protein 1 light chain 3 (LC3) is the autophagy marker in mammalian cells currently. LC3 is considered to be more specific autophagy diagnosis index involved in the formation of autophagy body [24]. In some diseases, autophagy is seen as an adaptive response to stress, while autophagy promotes cell death in others [25]. In our previous study [18], LC3 has been found to be expressed in SCC and adjacent normal tissues, but its expression in SCC was significantly lower than that in adjacent normal tissues, and its low expression was correlated with thickness of tumor tissues, TNM stage, histologic type and lymph node metastasis, and was negative correlation with the expression of apoptosis related genes. In this study, we also used LC3 to monitor the autophagy formation, and the result demonstrate that autophagy in skin cancer can be trigged ALA-PDT.

3-MA is an autophagy inhibitor and it inhibits autophagy by blocking autophagosome formation through the inhibition of class III phosphatidylinositol 3-kinases (PI3K) [26]. 3-MA has been implicated in cancer therapy [27], such as 3-MA enhanced antitumor effect of colorectal cancer [28] and lung cancer [29], and enhanced SCC radiation sensitization [30]. The chemotherapy drug, 5-FU is a DNA/RNA synthesis inhibitor which interrupts nucleotide synthetic by inhibiting thymidylate synthase in tumor cells. 5-FU has been usually used for anticancer in clinical [31]. We also found that 3-MA can enhance the chemotherapy sensitivity based on 5-FU in our previous study [18]. In this study, we try to understand whether 3-MA or 5-FU can enhance the efficacy of PDT, thus the combination treatment of ALA-PDT with 3-MA or 5-FU in skin cancer cells was used. In the study, we found that with

the treatments of ALA-PDT combined with or without 3-MA or 5-FU, the cell apoptosis was induced at different levels, especially, ALA-PDT combined with 3-MA or 5-FU treated cells. In all ALA-PDT treated cells, the expression levels of LC3 increased, MDC staining and transmission electron microscopy showed that the morphology change of cells with autophagy characteristics. Further study showed that the apoptotic genes Bax increased while Bcl2 decreasd, and survival gene Survivin decreased significantly with the treatment of ALA-PDT combined with or without 3-MA or 5-FU, also the apoptotic genes Caspase 3, 8 and 9 were also activated.

In conclusion, the proliferation of A431 and A375 cells were suppressed, while the apoptosis were induced by ALA-PDT and the effects can be enhanced by 3-MA or 5-FU pretreated, indicates that autophagy regulation may be the key role in the ALA-PDT process. Suggesting that ALA-PDT can enhance the anti-tumor effects of chemotherapy, and the combination therapy may be a strategy for treatment of non-melanotic cutaneous cancers or melanoma.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Acknowledgements

This work was supported by grants from the Science Foundation of Nantong City, Jiangsu Province, China (No. MS12017015-7) and Natural Science Foundation of China Youth Fund (No. 81703099).

References

[1] A. Kolk, K.D. Wolff, R. Smeets, et al., Melanotic and non-melanotic malignancies of the face and external ear - A review of current treatment concepts and future options, Cancer Treat. Rev. 40 (2014) 819–837.
[2] V. Parekh, J.T. Seykora, Cutaneous squamous cell carcinoma, Clin. Lab. Med. 37 (2017) 503–525.
[3] C. Cerchia, A. Lavecchia, Small molecule drugs and targeted therapy for melanoma: current strategies and future directions, Curr. Med. Chem. 24 (2017) 2312–2344.
[4] M.J. Veness, Advanced non melanoma skin cancers of the head and neck: an overview on management, Cancer Forum 30 (2006) 195–201.
[5] C.C. Ramirez, D.G. Federman, R.S. Kirsner, Skin cancer as an occupational disease: the effect of ultraviolet and other forms of radiation, Int. J. Dermatol. 44 (2005) 95–100.
[6] L.V. Vinicius de, C. Scapulatempo, N.M. Perpetuo, et al., Prognostic and risk factors in patients with locally advanced cutaneous squamous cell carcinoma of the trunk and extremities, J. Skin Cancer 2011 (2011) 420796.
[7] M.B. Visch, B. Kreike, M.P. Gerritsen, Long-term experience with radiotherapy for the treatment of non-melanoma skin cancer, J. Dermatolog. Treat. 20 (2019) 1–19.
[8] M.E. Menezes, S. Talukdar, S.L. Wechman, et al., Prospects of gene therapy to treat melanoma, Adv. Cancer Res. 138 (2018) 213–237.
[9] H. Abrahamse, M.R. Hamblin, New photosensitizers for photodynamic therapy, Biochem. J. 473 (2016) 347–364.
[10] A.M. Rkein, D.M. Ozog, Photodynamic therapy, Dermatol. Clin. 32 (2014) 415–425.
[11] H. Abrahamse, C.A. Kruger, S. Kadanyo, et al., Nanoparticles for advanced photodynamic therapy of Cancer, Photomed. Laser Surg. 35 (2017) 581–588.
[12] S. Battah, R.C. Hider, A.J. MacRobert, et al., Hydroxypyridinone and 5-Aminolaevulinic acid conjugates for photodynamic therapy, J. Med. Chem. 60 (2017) 3498–3510.
[13] U. Chilakamarthi, L. Giribabu, Chem Rec. Photodynamic Therapy: Past, Present and Future. 17 (2017) 775–802.
[14] S.R. Lucena, N. Salazar, T. Gracia-Cazaña, et al., Combined treatments with photodynamic therapy for non-melanoma skin Cancer, Int. J. Mol. Sci. 16 (2015) 25912–25933.
[15] J.Y. Guo, E. White, Autophagy, metabolism, and Cancer, Cold Spring Harb. Symp. Quant. Biol. 81 (2016) 73–78.
[16] E. Morselli, L. Galluzzi, O. Kepp, et al., Anti- and pro-tumor functions of autophagy, Biochim. Biophys. Acta 1793 (2009) 1524–1532.
[17] M. Hoare, A.R. Young, M. Narita, Autophagy in cancer: having your cake and eating it, Semin. Cancer Biol. 21 (2011) 397–404.
[18] L. Chen, L. Zhang, J. Zhang, et al., Autophagy in human skin squamous cell carcinoma: inhibition by 3-MA enhances the effect of 5-FU-induced chemotherapy sensitivity, Oncol. Rep. 34 (2015) 3147–3155.
[19] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, Methods. 25 (2001) 402–408.
[20] J.D. Bahner, J.S. Bordeaux, Non-melanoma skin cancers: photodynamic therapy, cryotherapy, 5-fluorouracil, imiquimod, diclofenac, or what? Facts and controversies, Clin. Dermatol. 31 (2013) 792–798.
[21] H. Wang, J. Li, T. Lv, et al., Therapeutic and immune effects of 5-aminolevulinic acid photodynamic therapy on UVB-induced squamous cell carcinomas in hairless mice, Exp. Dermatol. 22 (2013) 362–363.
[22] A. Brech, T. Ahlquist, R.A. Lothe, et al., Autophagy in tumour suppression and promotion, Mol. Oncol. 3 (2009) 366–375.
[23] N.S. Katheder, R. Khezri, F. O'Farrell, et al., Microenvironmental autophagy promotes tumour growth, Nature. 541 (2017) 417–420.
[24] H. Ito, S. Daido, T. Kanzawa, et al., Radiation-induced autophagy is associated with LC3 and its inhibition sensitizes malignant glioma cells, Int. J. Oncol. 26 (2005) 1401–1410.
[25] A.S. Patel, L. Lin, A. Geyer, et al., Autophagy in idiopathic pulmonary fibrosis, PLoS One 7 (2012) e41394.
[26] Y.T. Wu, H.L. Tan, G. Shui, et al., Dual role of 3-methyladenine in modulation of autophagy via different temporal patterns of inhibition on class I and III phosphoinositide 3-kinase, J. Biol. Chem. 285 (2010) 10850–10861.
[27] H. Cheong, C. Lu, T. Lindsten, et al., Therapeutic targets in cancer cell metabolism and autophagy, Nat. Biotechnol. 30 (2012) 671–678.
[28] Y. Wu, Y. Dong, G.L. Zhao, et al., Inhibition of autophagy by 3-MA promotes hypoxia-induced apoptosis in human colorectal cancer cells, Eur. Rev. Med. Pharmacol. Sci. 23 (2019) 1047–1054.
[29] X. Xue, X. Wu, L. Wang, et al., Suppressing autophagy enhances disulfiram/copper-induced apoptosis in non-small cell lung cancer, Eur. J. Pharmacol. 827 (2018) 1–12.
[30] Y.S. Chen, H.X. Song, Y. Lu, et al., Autophagy inhibition contributes to radiation sensitization of esophageal squamous carcinoma cells, Dis. Esophagus 24 (2011) 437–443.
[31] J.J. Lee, J.H. Beumer, E. Chu, Therapeutic drug monitoring of 5-fluorouracil, Cancer Chemother. Pharmacol. 78 (2016) 447–464.