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CD47 and *SIGLEC15* Immune Checkpoint Genes Expression and Apoptotic Pathway Activation Following Photodynamic Therapy with Zinc Phthalocyanine in the B-CPAP Thyroid Cancer Cell Line

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Abstract

Background & Objectives: Thyroid cancer is one of the most prevalent endocrine malignancies. Photodynamic therapy is an emerging minimally invasive therapeutic modality that employs a photosensitizer in conjunction with light irradiation to induce targeted cytotoxicity. The present study was designed to evaluate the effects of zinc phthalocyanine-mediated photodynamic therapy on the expression of immune checkpoint genes *CD47* and *SIGLEC15*, as well as key genes involved in apoptotic pathways, in papillary thyroid cancer cells.

Materials & Methods: The human papillary thyroid cancer cell line B-CPAP was exposed to various concentrations of zinc phthalocyanine and subsequently irradiated using a 675 nm diode laser at a fluence of 24 J/cm². Cellular viability, reflecting mitochondrial metabolic activity, was assessed using the MTT assay. The relative mRNA expression levels of *CD47*, *SIGLEC15*, *Caspase-3*, *Caspase-9* and *Bcl-2* genes were quantified using quantitative real-time polymerase chain reaction.

Results: Zinc phthalocyanine-mediated photodynamic therapy markedly reduced the viability of B-CPAP cells. The treatment activated the intrinsic apoptotic pathway, as demonstrated by the significant upregulation of *Caspase-3* and *Caspase-9* transcripts alongside the downregulation of Bcl-2 expression. In addition, a statistically significant reduction in the mRNA expression levels of the immune checkpoint molecules *CD47* and *SIGLEC15* was observed following treatment.

Conclusion: The findings of this study provide novel evidence that zinc phthalocyanine-mediated photodynamic therapy simultaneously induces apoptosis while downregulating the critical immune checkpoints *CD47* and *SIGLEC15* in thyroid cancer cells. This dual mechanism of action underscores the therapeutic potential of photodynamic therapy as an effective strategy for enhancing antitumor efficacy through both direct cytotoxic effects and the potential mitigation of immune evasion.

Keywords: Thyroid cancer, Photodynamic therapy, Zinc phthalocyanine, Immune checkpoints, Apoptosis

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Introduction

Thyroid cancer (TC) is the most common endocrine malignancy, with a global incidence that has steadily increased (1). Although the

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majority of cases, approximately 90%, are classified as well-differentiated papillary or follicular carcinomas with a favorable prognosis and a 5 year survival rate exceeding 97% (2), a substantial clinical challenge persists in advanced, recurrent, or treatment-refractory disease (3). By 2022, TC had become the seventh most common malignancy worldwide, underscoring its considerable public health burden (4).

Surgical resection remains the primary and most effective treatment for malignant TC and is frequently complemented by adjuvant modalities, including radioactive iodine ablation, hormone therapy, and, in selected cases, external beam radiation therapy or chemotherapy (4, 5). Despite their clinical efficacy, these conventional approaches are often invasive and lack specificity, thereby increasing the risk of long-term adverse effects and compromising patients' quality of life. Accordingly, there is a compelling need to develop non-invasive, targeted therapeutic strategies capable of selectively eradicating tumor cells while preserving healthy tissues (6, 7).

Photodynamic therapy (PDT) has emerged as a promising modality that fulfills these criteria. PDT utilizes a photosensitizing agent which, upon activation by light of a specific wavelength, generates cytotoxic reactive oxygen species. This process selectively induces apoptosis in malignant cells. Importantly, both the photosensitizer and the light source are considered individually safe, resulting in minimal collateral damage to surrounding normal tissues (8, 9). Beyond its direct cytotoxic effects, PDT has been shown to stimulate the host immune response, thereby further contributing to its antitumor activity. Nevertheless, the precise molecular mechanisms through which PDT modulates the tumor immune microenvironment, particularly in TC, remain incompletely elucidated and warrant further investigation.

A fundamental mechanism underlying tumor immune evasion involves the overexpression of immune checkpoint molecules. These regulatory proteins, including *SIGLEC15* and *CD47*, normally function to maintain immune homeostasis and prevent autoimmunity; however, they are frequently exploited by tumor cells to suppress antitumor immunity (10, 11). *SIGLEC15*, which is predominantly expressed on myeloid cells and osteoclasts, has been implicated in the establishment of an immunosuppressive tumor microenvironment (12). In contrast, *CD47*, commonly overexpressed on tumor cells, acts as a "do not eat me" signal that inhibits macrophage-mediated phagocytosis, thereby enabling cancer cells to evade innate immune surveillance. Consequently, targeting these immune checkpoints has become a central strategy in contemporary cancer immunotherapy (13).

Although the roles of these checkpoint molecules have been well characterized in several malignancies, their expression patterns, regulatory mechanisms, and therapeutic relevance in TC, particularly in the context of non-immunotherapeutic interventions such as PDT, remain poorly defined. This gap in knowledge raises an important question: can PDT, given its dual cytotoxic and immunostimulatory properties, directly modulate the expression of key immune evasion molecules such as *CD47* and *SIGLEC15* in TC cells?

Addressing this question is essential for elucidating the full mechanistic spectrum of PDT and for guiding the rational design of combination therapeutic strategies. Accordingly, the present study aims to bridge these two emerging fields by investigating the effects of zinc phthalocyanine (ZnPc)-mediated PDT on the expression of the immune checkpoint genes *SIGLEC15* and *CD47*, in parallel with key apoptotic markers, in papillary TC cells. We hypothesize that the immunomodulatory effects of PDT extend to the transcriptional downregulation of these immune evasion pathways, thereby revealing a novel dual mechanism of action.

Using an in vitro experimental model, this study seeks to provide the first direct evidence of

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this interaction in TC, to elucidate a previously uncharacterized mechanism underlying PDT, and to explore its potential as a non-invasive therapeutic approach that may be employed either as monotherapy or in combination with immunotherapeutic agents. Given the early stage of clinical application of PDT in TC, these findings are expected to offer a robust mechanistic foundation for future preclinical and clinical investigations aimed at improving outcomes in challenging cases.

Material and Methods

In Silico Analysis of Immune Checkpoints in Thyroid Cancer

This study was conducted in two integrated phases, comprising a comprehensive bioinformatic analysis followed by experimental validation. The bioinformatic phase began with the acquisition of RNA sequencing data from The Cancer Genome Atlas, a publicly accessible and ethically compliant repository. Transcriptomic profiles of thyroid carcinoma tissues were compared with those of normal thyroid tissues.

A curated panel of immune checkpoint genes, identified through an extensive review of the literature, was systematically analyzed within this dataset. Differential gene expression analysis was performed using R software, employing the Limma package to identify genes exhibiting statistically significant changes in expression. Immune checkpoint genes that were significantly overexpressed were subsequently selected for further analysis, and their correlations with a panel of apoptosis-related genes were assessed using the OncoDB platform.

For the experimental phase, the human B-CPAP thyroid carcinoma cell line was obtained from the Pasteur Institute of Iran Cell Bank. Cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and 1% penicillin streptomycin and were maintained in a humidified incubator at 37°C under an atmosphere containing 5% CO_2.

Photosensitizer

A stock solution of zinc phthalocyanine was prepared at a concentration of 5 mg/ml in dimethyl sulfoxide and subsequently diluted in RPMI-1640 medium to obtain a working solution of 100 µg/ml. The solution was sonicated for 15 minutes using a bath sonicator (Elma Transonic T420, Singen, Germany) to ensure homogeneity.

Serial dilutions were prepared from the working solution in RPMI-1640 medium to achieve a final concentration range of 0.0001 to 5 µg/ml for experimental application. The final concentration of dimethyl sulfoxide in all treatment media was maintained at or below 2% (v/v) to avoid solvent-induced cytotoxicity.

Light Source

B-CPAP cells were irradiated for 60 seconds using a 675 nm diode laser (Shenzhen Taiyong Technology, China) with an output power of 80 mW and an irradiance of 407 mW/cm². The technical specifications of the laser system are summarized in Table 1.

PDT Treatment

B-CPAP cells were assigned to five experimental groups to systematically evaluate the effects of photodynamic therapy. The first group consisted of untreated control cells that received neither zinc phthalocyanine nor light exposure. The second group, designated as the vehicle control, was treated with descending concentrations of dimethyl sulfoxide equivalent to those present in the zinc phthalocyanine solutions, without light irradiation, in order to control for solvent-related cytotoxicity. The third group, referred to as the zinc phthalocyanine dark control, was incubated with a concentration gradient of zinc phthalocyanine ranging from 0.0001 to 5 µg/ml under dark conditions to assess its intrinsic cytotoxic effects. The fourth group comprised cells exposed solely to laser irradiation at a fluence of 24 J/cm² in the absence of zinc phthalocyanine. The fifth group, representing the combined treatment condition, received both zinc phthalocyanine at

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Table 1. Primer sequence of genes used in qRT-PCR

Genes	Primer sequences
SIGLEC15	Forward: TGA CGA CCG CCG CTA CTT CT
	Reverse: ACT GGG CAG CAC CGA GAT GTT
CD47	Forward: GAC ACT GTC GTC ATT CCA TGC
	Reverse: GGG ACA GTG GAC TTG TTT AGA G
Bcl2	Forward: CCTGTGGATGACTGAGTACC
	Reverse: GAGACAGCCAGGAGAAATCA
Caspase-9	Forward: CCGGAATCCTGCTTGGGTATC
	Reverse: CATCGGTGCATTTGGCATGTA
Caspase-3	Forward: TGTCATCTCGCTCTGGTACG
	Reverse: AAATGACCCCTTCATCACCA
18SrRNA	Forward: ACCCGTTGAACCCCATTCGTGA
	Reverse: GCCTCACTAAACCATCCAATCGG

concentrations between 0.0001 and 5 µg/ml and subsequent laser irradiation at 24 J/cm².

For the relevant groups, cells were incubated with zinc phthalocyanine for 24 hours, after which they were washed twice with phosphate buffered saline and irradiated under dim, sterile conditions using a 675 nm laser. A 60 second interval was maintained between irradiation cycles. Cellular responses to treatment were assessed 24 hours following irradiation.

MTT Assay

The photodynamic therapy protocol was applied uniformly across all experimental groups of B-CPAP cells. To evaluate cell viability, as reflected by mitochondrial metabolic activity, an MTT assay was conducted following treatment with a concentration gradient of zinc phthalocyanine ranging from 0.0001 to 5 µg/ml, both in the presence and absence of laser irradiation. Prior to treatment, cells were seeded and allowed to adhere overnight to ensure the formation of a stable monolayer. Twenty-four hours after treatment, the culture medium was replaced with an MTT solution at a concentration of 2 mg/ml and incubated for 4 hours. Subsequently, the resulting formazan crystals were dissolved by adding 100 µl of dimethyl sulfoxide to each well. Optical density was measured at 570 nm with background correction at 620 nm using an enzyme linked immunosorbent assay microplate reader.

Apoptosis Assays

Flow Cytometric Analysis of Apoptosis and Necrosis

The induction of apoptosis and necrosis following zinc phthalocyanine mediated photodynamic therapy was quantified using flow cytometry. Treated B-CPAP cells were harvested, resuspended, and stained with an ApoFlowEx FITC kit according to the manufacturer's instructions. Briefly, the cell suspension was incubated with 5 µl of Annexin V FITC and 5 µl of propidium iodide in 190 µl of binding buffer for 10 minutes at room temperature in the dark. Samples were subsequently analyzed by flow cytometry to distinguish viable cells (Annexin V negative and propidium iodide negative), early apoptotic cells (Annexin V positive and propidium iodide negative), late apoptotic cells (Annexin V positive and propidium iodide positive), and necrotic cells (Annexin V negative and propidium iodide positive).

Quantitative Real-Time PCR (qRT-PCR) Analysis

Total RNA was extracted from treated B-CPAP cells 24 hours after treatment using Ribo Ex LS RNA solution. Complementary DNA was synthesized from purified RNA using a commercial cDNA synthesis kit. Quantitative real time polymerase chain

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reaction was performed using a StepOne Plus system with SYBR Green PCR Master Mix. The expression levels of the target genes *CD47*, *SIGLEC15*, *Caspase-9*, *Caspase-3*, and Bcl-2 were analyzed, with 18S rRNA serving as the endogenous reference gene for normalization. Gene specific primer sequences are provided in Table 1. Relative gene expression levels were calculated using the comparative ΔΔCt method.

Statistical Analysis

All experiments were conducted with at least three independent replicates. Data are presented as the mean ± standard deviation. Statistical analyses were performed using GraphPad Prism software, Version 6. Comparisons between groups were carried out using a two tailed Student's t test. A p value of less than 0.05 was considered statistically significant.

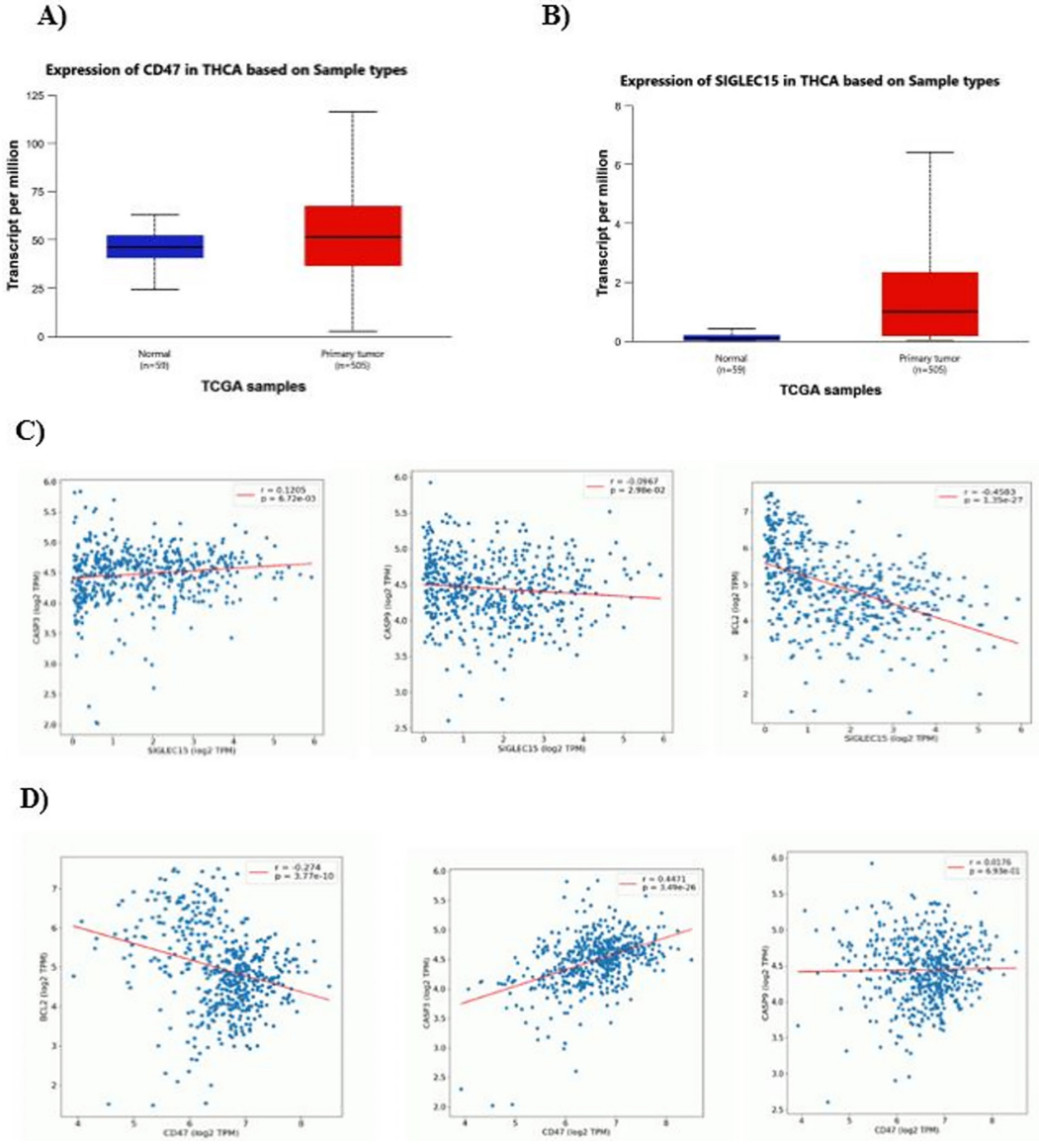


Figure 1. Expression levels of *CD47*, *SIGLEC15*, apoptosis-related genes, and immune checkpoint molecules. (A, B) Significant differences in the expression of *CD47* and *SIGLEC15* between normal and thyroid cancer samples derived from TCGA. (C) Association between apoptosis-related genes (Bcl-2, *Caspase-9*, and *Caspase-3*) and the immune checkpoint *SIGLEC15*, based on OncoDB analysis. (D) Association between apoptosis-related genes (Bcl-2, *Caspase-9*, and *Caspase-3*) and the immune checkpoint *CD47*, based on OncoDB analysis.

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Results

Significance of *CD47* and *SIGLEC15* in Thyroid Cancer Patients.

Bioinformatic analysis of data obtained from The Cancer Genome Atlas, conducted using the R programming environment and the Limma package for normalization, revealed a significant upregulation of the immune checkpoint genes *CD47* and *SIGLEC15* in thyroid carcinoma tissues compared with normal thyroid tissues. This differential expression, visualized using the UALCAN platform (Figure 1A and 1B), indicates a potential role for these molecules in mediating immune evasion during thyroid tumorigenesis. Further analysis using the OncoDB database demonstrated significant correlations between the expression levels of these immune checkpoint genes and key regulators of apoptosis, including Bcl-2, *Caspase-9*, and *Caspase-3* (Figure 1C and 1D). These findings suggest the existence of a functional interplay between immune checkpoint regulation and apoptotic signaling pathways.

The Consequences of ZnPc-PDT on the Cell Viability of B-CPAP Cells

The cytotoxic effects of zinc phthalocyanine mediated photodynamic therapy on B-CPAP cells were evaluated by measuring cell viability following exposure to a gradient of zinc phthalocyanine concentrations ranging from 0.0001 to 5 µg/ml, followed by laser irradiation at 675 nm with a fluence of 24 J/cm². The MTT assay demonstrated that treatment with zinc phthalocyanine alone, the dimethyl sulfoxide vehicle, or laser irradiation alone did not result in statistically significant cytotoxicity compared with the untreated control group ($p > 0.05$). In contrast, the combined treatment consisting of zinc phthalocyanine and irradiation produced a marked and dose dependent reduction in cell viability ($p < 0.0001$; Figure 2). Nonlinear regression analysis revealed that the half maximal inhibitory concentration (IC_{50}) of zinc phthalocyanine mediated photodynamic therapy at 24 hours post treatment was 0.1 µg/ml.

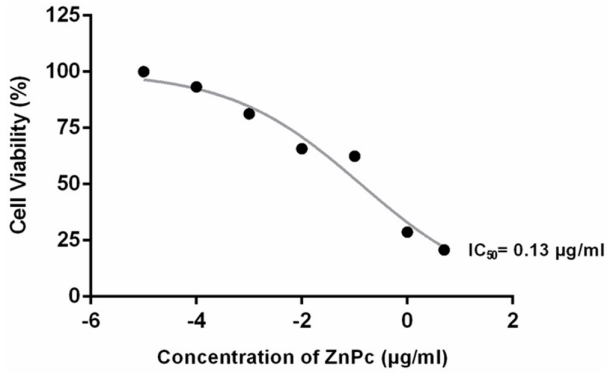


Figure 2. Cytotoxic effects of zinc phthalocyanine mediated photodynamic therapy on B-CPAP cells and determination of the IC_{50} value at 24 hours post treatment ($P<0.0001$****).

The Consequences of ZnPc-PDT on Apoptosis/ Necrosis

Exposure of B-CPAP cells to zinc phthalocyanine mediated photodynamic therapy at a fluence of 24 J/cm² resulted in a significant increase in apoptosis compared with the control group ($p < 0.0001$; Figure 3A). Further analysis of key apoptotic regulators indicated that cell death was predominantly mediated via the intrinsic apoptotic pathway. Specifically, treatment with zinc phthalocyanine mediated photodynamic therapy led to a pronounced upregulation of the initiator caspase, *Caspase-9*, and its downstream effector, *Caspase-3* ($p < 0.0001$). Concurrently, a significant downregulation of the anti apoptotic protein Bcl-2 was observed ($p < 0.0001$; Figure 3B).

Discussion

Despite substantial advances in the management of thyroid cancer, tumor recurrence, metastasis, and the emergence of treatment resistance continue to pose major clinical challenges. Immune checkpoint pathways play a central role in mediating these processes by enabling tumor cells to evade immune surveillance. Consequently, the targeted modulation of these pathways has emerged as a promising strategy for the development of novel therapeutic approaches in thyroid cancer (14, 15).

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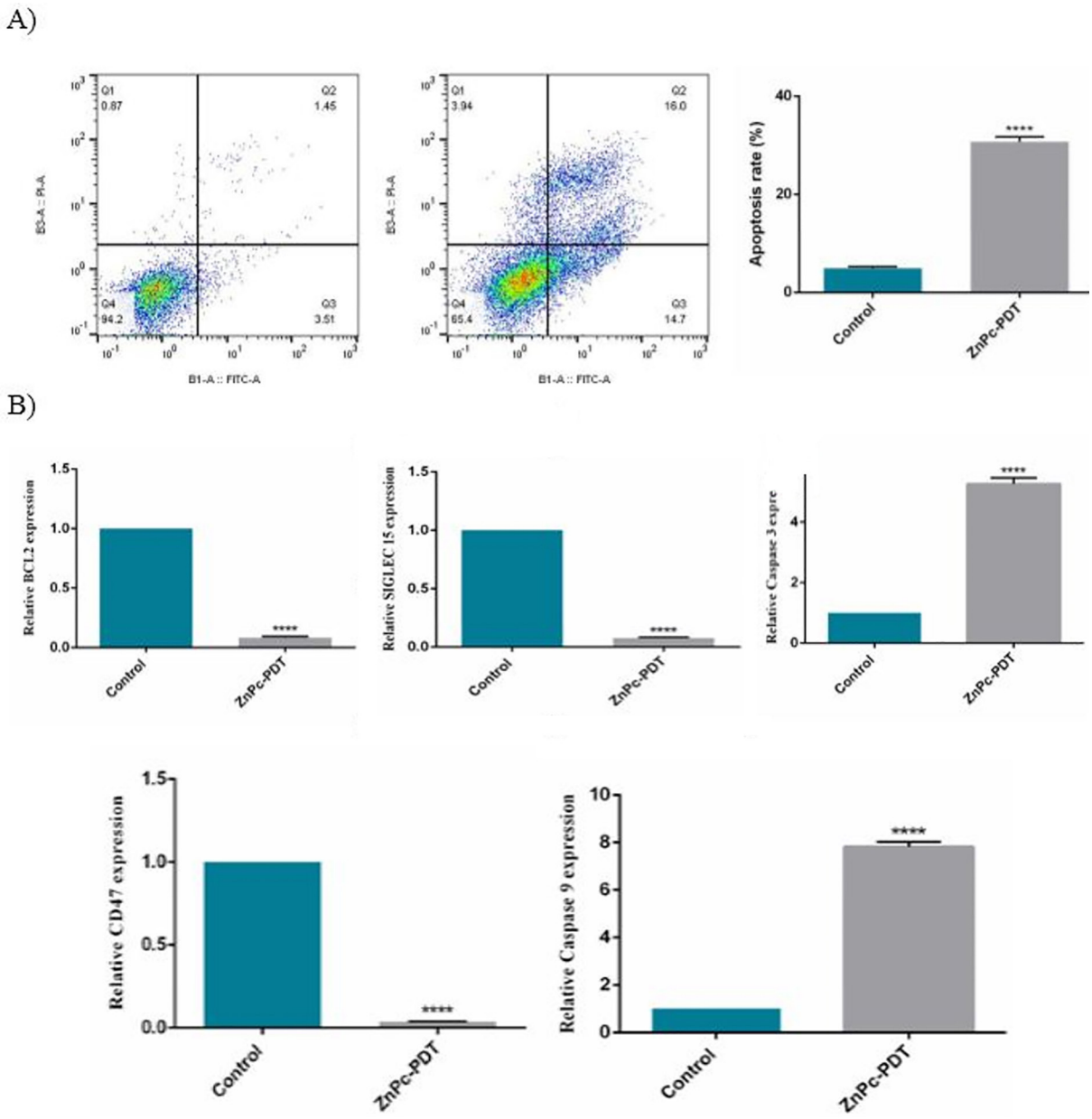


Figure 3. A) Shows the results obtained from flow cytometry, which showed that the rate of apoptosis and necrosis increased in the group treated with ZnPc-PDT. **B).** shows the results obtained from qRT-PCR that the expression levels of cas3 and cas9 genes increased after treatment with ZnPc-PDT in B-CPAP TC cells. Also, the expression levels of *Bcl-2*, *CD47*, and *SIGLEC15* genes decreased. The outcomes are expressed as mean +_ SD (n = 3); **** $p < 0.0001$, **** $p < 0.0001$ against controller.

The findings of the present study demonstrate that zinc phthalocyanine mediated photodynamic therapy effectively suppresses thyroid cancer cell proliferation while inducing apoptosis. These observations are consistent with an expanding body of evidence supporting the efficacy of zinc phthalocyanine based photodynamic therapy across a range of malignancies. Previous studies have reported that this therapeutic approach significantly reduces cell viability (16), induces mitochondrial mediated apoptosis and cell cycle arrest (17), and inhibits angiogenesis while

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enhancing *Caspase-3* activity (18). Moreover, structural modifications of zinc phthalocyanine aimed at improving water solubility have been shown to enhance its antitumor efficacy, particularly in breast cancer models (19). Collectively, these findings underscore the broad therapeutic potential of zinc phthalocyanine as a photosensitizer and corroborate our results in the B-CPAP thyroid cancer cell line. In particular, our data confirm that zinc phthalocyanine mediated photodynamic therapy at a fluence of 24 J/cm² activates the intrinsic apoptotic pathway, as evidenced by the upregulation of *Caspase-9* and *Caspase-3* and the concomitant downregulation of Bcl-2.

In addition to its direct cytotoxic effects, photodynamic therapy appears to exert significant immunomodulatory influences. It has been suggested that photodynamic therapy may enhance antitumor immunity, in part, by reducing the expression of *SIGLEC15*, which is known to inhibit NF-κB and NFAT signaling pathways, thereby suppressing cytokine production and T cell proliferation (20). Similarly, photodynamic therapy may counteract macrophage inhibition by disrupting *CD47* mediated signaling. Given that *CD47* interacts with signal regulatory protein alpha to inhibit macrophage activation, its downregulation may restore phagocytic activity and promote innate immune responses (13).

Importantly, the most novel and significant finding of the present study lies in the observation that zinc phthalocyanine mediated photodynamic therapy concurrently downregulates the expression of the immune checkpoint molecules *SIGLEC15* and *CD47* in thyroid cancer cells. This finding suggests the existence of a dual mechanism of action, encompassing both direct cytotoxicity and immune modulation.

Although the precise molecular mechanisms underlying this effect remain to be fully elucidated, several plausible explanations can be proposed. First, the oxidative stress generated by photodynamic therapy, together with damage to intracellular organelles such as the endoplasmic reticulum, may activate integrated stress response pathways, thereby inducing a global reprogramming of gene expression that shifts cellular priorities away from immune evasion. Second, photodynamic therapy is known to induce immunogenic cell death, characterized by the surface exposure of calreticulin and the release of adenosine triphosphate and high mobility group box 1 protein (21, 22). These signals promote phagocytosis and may functionally counteract the "do not eat me" signal mediated by *CD47*, potentially establishing a feedback mechanism that contributes to its downregulation.

With respect to *SIGLEC15*, its expression is closely associated with myeloid cell activity and the cytokine milieu within the tumor microenvironment (12, 23-25). The capacity of photodynamic therapy to alter the tumor microenvironment through the release of damage associated molecular patterns and pro inflammatory cytokines may shift local signaling dynamics, thereby reducing the expression of *SIGLEC15*.

Immune checkpoint inhibitors, including monoclonal antibodies targeting PD-1 and PD-L1, have been associated with thyroid dysfunction as a consequence of immune modulation. These agents function by releasing inhibitory constraints on T cells, thereby enhancing antitumor immune responses. However, this heightened immune activation is accompanied by an increased risk of immune related adverse events, arising from off target inflammation of healthy tissues due to autoimmune mechanisms.

In most cases, immune related adverse events initially affect the liver, gastrointestinal tract, skin, and endocrine system. Among endocrine complications, thyroid dysfunction represents one of the most frequently observed manifestations, particularly in patients receiving anti PD-1 monoclonal antibody therapies. The clinical burden associated with these adverse

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events, together with the complexities involved in their management, has shifted research priorities toward the development of therapeutic strategies that mitigate immune related toxicity (26).

Within this context, the ability to locally downregulate immune checkpoints such as *CD47* and *SIGLEC15* through photodynamic therapy assumes particular significance. Unlike systemic immune checkpoint inhibition, a localized modality such as photodynamic therapy offers a strategic advantage by targeting immune evasion mechanisms directly at the tumor site while minimizing systemic immune activation, thereby potentially reducing the incidence of immune related adverse events.

Consistent with this perspective, Cramer et al. (2020) reported that PD-L1 expression in tumor cells was reduced 24 hours following photodynamic therapy compared with untreated controls. Notably, photodynamic therapy can also modulate the tumor microenvironment in a manner that supports immune activation. By inducing the release of inflammatory mediators, photodynamic therapy promotes both innate and adaptive immune responses in addition to its direct cytotoxic effects. These mediators facilitate antigen uptake and presentation by antigen presenting cells, thereby enhancing T cell priming within lymphoid tissues (27).

Furthermore, photodynamic therapy has been associated with a substantial increase in intratumoral neutrophil infiltration, which may account for more than 30 percent of the viable cell population within 24 hours after treatment. These neutrophils contribute to the regulation of subsequent immune responses by modulating chemotactic signals that influence the recruitment and maturation of dendritic cells (28).

In a recent study, Ye et al. (2025) demonstrated that combined photodynamic and photothermal therapies induce immunogenic cell death and PANoptosis in tumor cells. This dual modality not only suppressed tumor growth and metastasis in murine models but also elicited a robust immune response, as evidenced by increased infiltration of CD8 positive T cells, CD4 positive T cells, and dendritic cells, alongside a reduction in immunosuppressive populations such as myeloid derived suppressor cells and regulatory T cells (29).

The immunomodulatory capacity of photodynamic therapy, now extended by our findings to include the downregulation of *CD47* and *SIGLEC15*, further strengthens the rationale for combining photodynamic therapy with systemic immunotherapy. In this framework, photodynamic therapy may function as an in situ vaccine by reducing tumor burden, attenuating local immune suppression, and establishing a pro inflammatory tumor microenvironment. Such a primed microenvironment could act synergistically with subsequent immune checkpoint inhibition, thereby enhancing systemic antitumor immunity while permitting the use of lower and less toxic doses of systemic agents (27, 29).

Accordingly, this combined approach represents a practical and adaptable therapeutic strategy. Photodynamic therapy may be employed to target and reprogram the primary tumor, thereby enhancing the responsiveness of residual malignant cells to immune checkpoint blockade. In conclusion, the present study demonstrates that zinc phthalocyanine mediated photodynamic therapy functions not only as a potent inducer of apoptosis in thyroid cancer cells but also as a regulator of key immune evasion pathways. The dual capacity to promote tumor cell death while downregulating *CD47* and *SIGLEC15* positions photodynamic therapy as a multifaceted therapeutic modality. Future investigations should focus on validating these findings in in vivo models, elucidating the molecular mechanisms underlying checkpoint modulation, and exploring combinatorial treatment strategies integrating photodynamic therapy with immunotherapeutic agents to improve outcomes in patients with advanced or refractory thyroid cancer.

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Conclusions

Immune checkpoints constitute a central mechanism of resistance in cancer therapy, underscoring the importance of targeting these pathways. The findings of the present study indicate that reduced expression of *SIGLEC15* and *CD47* is significantly associated with disease progression in thyroid cancer, highlighting their potential utility as prognostic biomarkers. Our results demonstrate that zinc phthalocyanine mediated photodynamic therapy at an irradiation dose of 24 J/cm² significantly decreases tumor cell viability. Furthermore, this treatment activates the intrinsic apoptotic pathway, as evidenced by the downregulation of Bcl-2 and the upregulation of *Caspase-3* and *Caspase-9*. In addition, we propose that zinc phthalocyanine mediated photodynamic therapy may enhance antitumor immunity in thyroid cancer by reducing the expression of *SIGLEC15* and *CD47*, thereby potentially limiting disease progression. Collectively, these findings support the therapeutic potential of photodynamic therapy as both a cytotoxic and immunomodulatory intervention.

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Conflict of Interest

The authors declare no competing interests.

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Ethical Consideration

Ethical approval was obtained from the Clinical Research Ethics Committee of Islamic Azad University, Tabriz Branch, under approval number IR.IAU.TABRIZ.REC.1403.412, dated 30 October 2024.

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Author Contributions

Concept/Design: MP, AJS, HT; Data acquisition: GF, SSSO; Data analysis and interpretation: MP, AJS; Drafting manuscript: GF, SSSO; Critical revision of manuscript: HT; Final approval and accountability: MP; Technical or material support: MP, AJS, HT; Supervision: MP, HT.

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