


Scientific paper

# Effect of Biphenyl Derivative of Coumarin Compounds Photodynamic Therapy on The Expression of Carcinoma-Associated Genes

Asiye Gok Yurttas<sup>1,\*</sup> , Tugba Elgun<sup>2,6</sup>, Burcin Erkal Cam<sup>3</sup>, Melike Kefeli<sup>4</sup> and Kamil Cinar<sup>5</sup>

<sup>1</sup> Department of Biochemistry, Faculty of Pharmacy, Istanbul Health and Technology University, Istanbul, Türkiye

<sup>2</sup> Medical Biology, Faculty of Medicine, Istanbul Biruni University, Istanbul, Türkiye

<sup>3</sup> Department of Molecular Biology and Genetics, Yildiz Technical University, Istanbul, Türkiye

<sup>4</sup> Molecular Biology and Genetics, Istanbul University, Istanbul, Türkiye

<sup>5</sup> Department of Physics, Faculty of Science, Gebze Technical University, Kocaeli, Türkiye

<sup>6</sup> Biruni University Research Center (B@MER), Biruni University, 34015, Istanbul, Türkiye

\* Corresponding author: E-mail: asiye.yurttas@istun.edu.tr

Phone/Fax: +90 533 280 87 07

Received: 08-22-2024

## Abstract

Photodynamic Therapy (PDT) is a cancer treatment. Singlet oxygen is produced as a result of the photochemical reaction between light, photosensitizer (PS), and molecular oxygen, which kills cells. Colon cancer, affecting 1.23 million people worldwide, often requires surgery but has high recurrence and metastasis rates. Photodynamic therapy (PDT) represents an alternative treatment for colon cancer. This study used MTT assays to evaluate cell viability and applied Zinc (II) Phthalocyanine (ZnPc) photosensitizers to the colorectal adenocarcinoma (HT-29) cell line to investigate cancer pathways via flow cytometry and q-PCR. The results showed that PDT with ZnPc significantly reduced cell viability in HT-29 cells and induced apoptosis at a rate of 53%. According to q-PCR results, CT values of ten out of thirty genes were significant, and their association with cancer was evaluated.

**Keywords:** Photodynamic therapy, HT-29 cancer cell, Coumarin, Phthalocyanine, Gene expression

## 1. Introduction

The second most significant cause of death in the world is cancer.<sup>1</sup> Popular cancer therapies include surgery, chemotherapy, radiation, and immunotherapy. However, numerous regulatory cell signaling pathways, such as cell cycle arrest, apoptosis, or migration, have been discovered to impair therapeutic effectiveness process. Cancer cells' cellular heterogeneity may restrict the treatment options available to treat the illness.<sup>2</sup> The vast heterogeneity in tumor cell populations at the patient and cell level is a significant barrier to effective cancer treatment.<sup>3</sup> Different cancer cells have different responses to therapy in terms of acquiring drug tolerance, surviving, and having the potential to spread. Subsets of hematological and solid tumors,

including breast, ovarian, lung, and lower gastrointestinal tract malignancies, have been found to evolve the multidrug-resistant genotype.<sup>3</sup>

The continuous accumulation of genetic and epigenetic changes is the hallmark of the complex colorectal cancer (CRC) illness.<sup>4</sup> The fourth most common cause of cancer-related deaths worldwide and the third most dangerous malignancy overall are CRCs.<sup>5</sup> Surgery, chemotherapy or radiotherapy are the standard curative therapies for CRC patients. However, these procedures have a lot of side effects and need a lot of recovery time. With the creation of fresh medications and therapeutic regimens, significant advancements in the therapy of CRC have been made.<sup>6,7</sup> However, because tumor cells are resistant to existing treatment approaches.

Due to its excellent specificity and selectivity, photodynamic therapy (PDT) is an appealing anticancer treatment.<sup>8</sup> PDT is a recognized medical procedure worldwide, including in Canada, Japan, Brazil, Chile, Argentina, the US, and the EU.<sup>9</sup> PDT is a minimally invasive treatment technique that has been clinically established and can selectively kill cancer cells.<sup>10</sup> PDT has the following benefits over other cancer therapy modalities: 1- Low side effect profile in the dark; 2- Because tissues differ physiologically, photosensitizers accumulate more in tumor tissues than in healthy tissues; 3- It can be used in conjunction with other treatments; 4- It can be used without surgery; 5- The effects are seen in 24 to 48 hours; and 6- The risk of cancer recurrence is low after PDT.<sup>11</sup> PDT is presently employed in numerous fields, such as ophthalmology, phot-immunotherapy, vascular targeting, and the treatment of cancer and acne.<sup>12</sup>

A wide range of biological actions, anti-tumor potential<sup>13</sup>, including antifungal, anticoagulant, vasodilator, estrogenic, dermal, photosensitizing, sedative, hypnotic, analgesic, antimicrobial, anti-inflammatory, anti-HIV, and anti-ulcer effects, are displayed by compounds with a coumarin moiety.<sup>14</sup> Phthalocyanines are used commercially as dyes and pigments in printing inks, coloring plastic and metal surfaces, laser technology, optical and electrical materials, photodynamic cancer therapy, and chemical sensors. Functional phthalocyanines are needed for the development of different reactions on phthalocyanine complexes. These two functional compounds, coumarins, and phthalocyanines, can be combined in a single compound by synthetic methods to obtain soluble-fluorescent phthalocyanines. According to the experimental results; the addition of coumarin derivatives to the peripheral positions of the phthalocyanine ring increased the fluorescence properties and solubility of phthalocyanines.<sup>14</sup> The original metal phthalocyanine, a new biphenyl derivative of coumarin compounds Zinc (II) Phthalocyanine (ZnPc), was synthesized.<sup>14</sup>

This article investigated the cytotoxicity of ZnPc against colorectal adenocarcinoma cell line (HT-29 cells). It has been demonstrated that PDT and particular photosensitizers (PSs) kills cancer cells.<sup>15</sup> The underlying mechanism of cell death induction must be identified to evaluate the effectiveness of PDT utilizing particular PSs. Therefore, in this study, we compare the effects of ZnPc on HT-29 cells and the cancer pathways to better understand the etiopathogenesis of human colorectal adenocarcinoma. This knowledge will facilitate innovative therapeutic approaches in the future.

## 2. Material and Methods

### 2. 1. Procedure for the Synthesis of ZnPc

In our previous study, biphenyl derivative of coumarin [7-(2,3-dicyanophenoxy)-3-biphenylcoumarin 2

(0.10 g, 0.227 mmol)] with metal salt [Zn(OAc)2.2H2O (0.01 g, 0.046 mmol)], and two drops of DBU in 3 mL dry hexanol in a sealed glass tube was heated at 180 °C and stirred for 30 h under an argon atmosphere. After cooling to room temperature, the mixture was treated with 10 mL ethanol. The obtained products were filtered off and washed first with hot water then ethanol, ethylacetate and dried.<sup>14</sup>

### 2. 2. Photodynamic Therapy Treatment of Cell and Cell Viability Assay

The HT-29 colorectal adenocarcinoma cell line from (ATCC, USA) was routinely cultured in Dulbecco's Modified Eagle's Medium (DMEM) with the addition of 10% heat-inactivated fetal bovine serum (FBS), 1% penicillin-streptomycin (Sigma, USA), and 37 °C in a humid environment with 5% CO<sub>2</sub>. Adherent monolayer cells were passaged at 70-80% confluence using trypsin EDTA (Sigma, USA). Each cell line was seeded onto 96-well plates with 100 µL of media per well (1x10<sup>4</sup> cells), and the wells were attached after 24 hours of incubation at 37 °C. Cells were gathered to perform viability tests. The HT-29 cells of 1 × 10<sup>4</sup> cell density were cultured for 24 h before being exposed to eight concentrations of ZnPc, as 0.5, 1, 3, 5, 10, 15, 20, and 40 µM prepared from the leading stocks of ZnPc, which were prepared according to literature.<sup>16</sup> The cells were first grown with PSs for 24 hours before being photosensitized with a diode laser at 660 nm with a fluence of 5.4 J/cm<sup>2</sup> for phototoxicity tests. The cells were then collected, and their vitality was assessed.<sup>16</sup> Each well's culture media was taken out, and the cells were then treated with ZnPc and rinsed with PBS. Using the MTT test kit, the cell viability (%) was calculated (Thiazolyl Blue Tetrazolium Bromide, Sigma Aldrich, Missouri, ABD, Cas: 298-93-1). HT-29 cell line was then cultured for 3 hours at 37 °C before being slowly rinsed in PBS with a pH of 7.4. A spectrophotometer (540nm) was used to determine the cell viability, which was then computed using the formula below: Viability = (Sample-Blank)/(Control-Blank). Three times during three different weeks, the experiments were repeated.<sup>18</sup>

### 2. 3. Flow Cytometry

At 24 hours after treatment with increasing doses of ZnPc, apoptosis, and necrosis were identified using a flow cytometric assay.<sup>17</sup> This was done using the Annexin V/7-Aminoactinomycin D (7-AAD) kit from Invitrogen/Biolegend in San Diego, California, USA. According to the manufacturer's recommendations, the HT-29 cell line (8 × 10<sup>5</sup> cells/well) was seeded in 6-well plates with 2 mL of medium and cultivated at 37 °C for 24 hours. The cells underwent two cold PBS washes after being collected into individual eppendorf tubes. After all cells were centrifuged at 1500 rpm for 5 minutes, the supernatant was gathered. After adding the annexin V binding buffer, cells

were counted at a density of  $10^6$  cells per milliliter. Cells were treated for 15 minutes at room temperature in the dark with 5  $\mu\text{L}$  of Annexin V and 5  $\mu\text{L}$  of 7-AAD. After 400  $\mu\text{L}$  of binding buffer was injected on ice, the percentages of apoptosis and necrosis were calculated by flow cytometry (BD AccuriTM C6 Plus) (Figure 1–2).

## 2. 4. RNA Isolation and Global Dna Methylation Assessment

Total RNA was isolated using a TRIzol reagent (Life Technologies, Carlsbad, CA, USA). Total RNA concentrations were determined using a Nanodrop 2000c (Thermo Fisher Scientific). cDNA was synthesized with Wonder RT- cDNA Synthesis kit (Euroclone, Milan, Italy). Following cDNA synthesis, the level of global DNA methylation was estimated using the Methyl flash™ Global DNA methylation (5-mC) ELISA Easy Kits (Epigentek Group Inc, USA) according to the manufacturer's protocol.

## 2. 5. qPCR

According to the manufacturer's recommendations, total RNA was extracted from cell and tumor tissue samples using the TRIzol reagent (Life Technologies, Carlsbad, CA, USA). Following reverse transcription, complementary DNA (cDNA) was produced and put through real-time PCR using the proper primers and SYBR Green Mix (Thermo Fisher Scientific). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control. Each experiment was performed in triplicate. Each data item was calculated using the  $2^{-\Delta\Delta\text{CT}}$  method (Table 1).<sup>19</sup>

## 2. 6. Gene Enrichment Analysis of Significant Genes

Protein-protein interactions (PPI) of the prominent genes between groups were performed using the STRING

tool. PPI analyses were visualized using the Cytoscape program. Pathway and Gene ontology analyses of these genes were also performed using both STRING and cancer hallmarks tool. The expression status of these genes in COAD data (Cancer vs. normal) was also evaluated using the UALCAN tool, which performs analyses using TCGA datasets. In addition, survival analysis of the prominent genes was performed using the TIMER 2.0 tool. P value < 0.05 was accepted as statistically significant.

## 2. 7. Statistical Analyses

Mean SD were the descriptive statistics used to report all values. Welch correction and a t-test without pairings were employed to compare two unpaired variables. ANOVA was used to analyze more than two parametric variables, and the Tukey-Kramer Multiple Comparisons Test was used for a post hoc analysis. The cutoff for significance in all statistical studies is fixed at  $P < 0.05$ . GraphPad Instat (GraphPad Software, San Diego, CA, USA) was used to conduct the analyses.

## 3. Results

### 3. 1. Cytotoxicity Studies and Phototherapeutic Effect of Znpc Photosensitizer *In Vitro*

According to the morphological examination, 20 $\mu\text{M}$  ZnPc significantly suppressed the cell growth of the HT-29 cell line when compared to the control groups. According to the MTT experiment, the effective dose of ZnPc, specifically for the HT-29 cell line, was 20 $\mu\text{M}$  ( $P^{****} < 0.001$ ). The increasing doses of ZnPc dramatically lowered the cell viability of HT-29 cells. These findings indicate a dose-dependent inhibition of cell viability by the ZnPc treatment in HT-29 cells. The concentrations between 0.5 and 10  $\mu\text{M}$

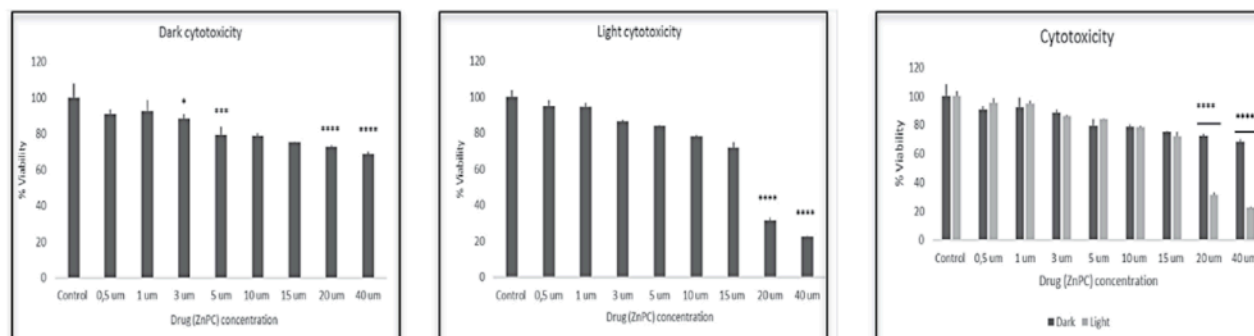


Figure 1. The cell viabilities (%) of HT29 cells calculated according to the results of MTT assay

Survival ratios of HT-29 cells incubated in ZnPc were analyzed using dark and light toxicity assays.  $****P < 0.001$  vs normal cells. All data shown are the mean  $\pm$  standard deviation of three experiments performed independently. \* $P < 0.05$ , \*\* $P < 0.01$  and  $***P < 0.001$  are the statistically significance levels. ns: not significant. 3 $\mu\text{M}$  vs. Control \* $P < 0,05$ ; 5  $\mu\text{M}$  vs. Control  $***P < 0,001$ ; 20 $\mu\text{M}$  vs. Control  $****P < 0,001$ ; 40 $\mu\text{M}$  vs. Control  $****P < 0,001$  analyzed by dark toxicity assays. 20 $\mu\text{M}$  vs. Control  $****P < 0,001$ ; 40 $\mu\text{M}$  vs. Control  $****P < 0,001$  analyzed by light toxicity assays. 20 $\mu\text{M}$  (dark) vs 20 $\mu\text{M}$  (light); 40 $\mu\text{M}$  (dark) vs 40 (light)  $****P < 0,001$  analyzed by dark and light toxicity assays.

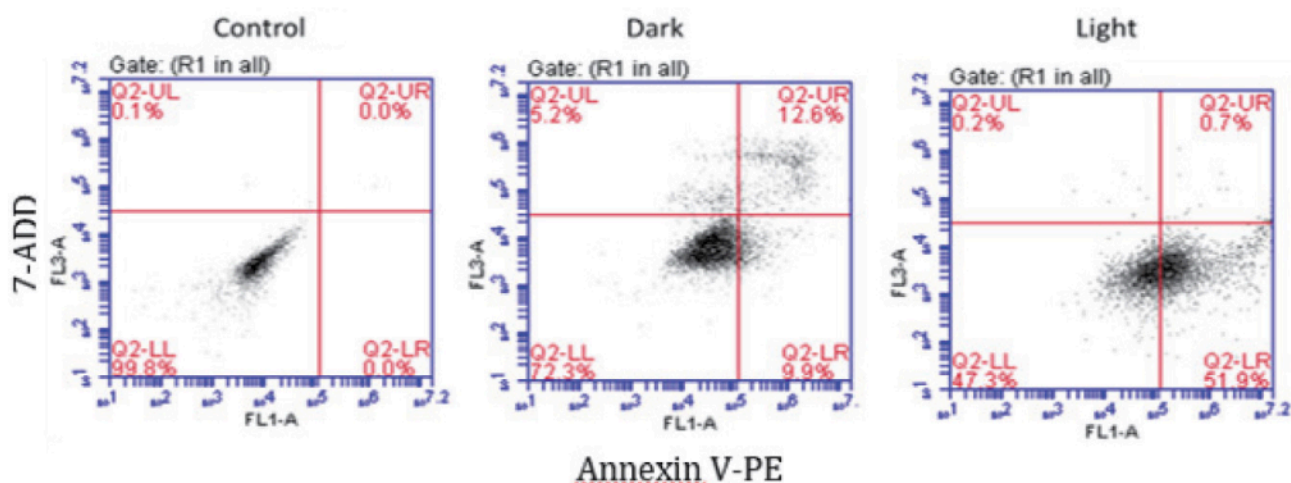


Figure 2. Analysis of Annexin-V/7AAD in HT-29 cells.

were also examined but did not give significant results in this range. One of the desirable characteristics of an efficient ZnPc is to have a high toxicity in the light and a low toxicity in the dark [17]. This was accomplished for ZnPc at a concentration of 20  $\mu\text{M}$ , per the MTT results from the current investigation. Statistical analysis used One-Way Analysis of Variance (ANOVA) and the Tukey-Kramer Multiple Comparison Test with Post-hoc Test. This concentration of ZnPc, showed minimal toxicity in the dark (75%,  $P^{***} < 0.001$ ), and their survival ratio decreased significantly (lower than 35%,  $P^{***} < 0.001$ ) under light irradiation. While ZnPc (at 20  $\mu\text{M}$  concentration) toxicity showed was showing minimal toxic effects in the dark, a high rate of toxicity was observed at 5.4  $\text{J}/\text{cm}^2$  light dose at the same concentration in the light (Figure1).

### 3. 2. Irradiation on HT-29 Cell

In our meticulously designed experimental configuration, we utilized a red laser diode emitting light at a central wavelength of 660 nm to irradiate a precisely defined area of 0.2  $\text{cm}^2$  on HT-29 cell samples. The laser's Gaussian beam profile played a crucial role in our calculations, as it excluded the tails, whose contribution to exposure fluence was deemed negligible. This meticulous approach ensured a high level of precision in assessing the intricate interactions between light and matter.

In a related experiment, we exposed HT-29 cells to a specific fluence using a continuous wave diode laser, following which we evaluated the subsequent biological responses after a 24-hour incubation period in fresh, photosensitizer-free media. This post-exposure evaluation allowed us to gain valuable insights into the longer-term effects of laser irradiation on cellular responses.

Our investigation focused on the dynamic interplay between ZnPc and HT-29 cells, maintaining a cell density  $10^4$ . The diode laser, with a power output of 5 mW and a central wavelength of 660 nm, delivered an irradiation

that deposited an energy density of 5.4  $\text{J}/\text{cm}^2$ . Notably, the study honed in on the energy absorption characteristics of ZnPc solutions, specifically at a concentration of 20  $\mu\text{M}$ . The results unveiled a remarkable finding: the solution of ZnPc at 20  $\mu\text{M}$  in a particular solvent exhibited the highest photo-toxicity among the examined conditions.

### 3. 3. Apoptotic Effect of ZnPc on HT-29 Cell

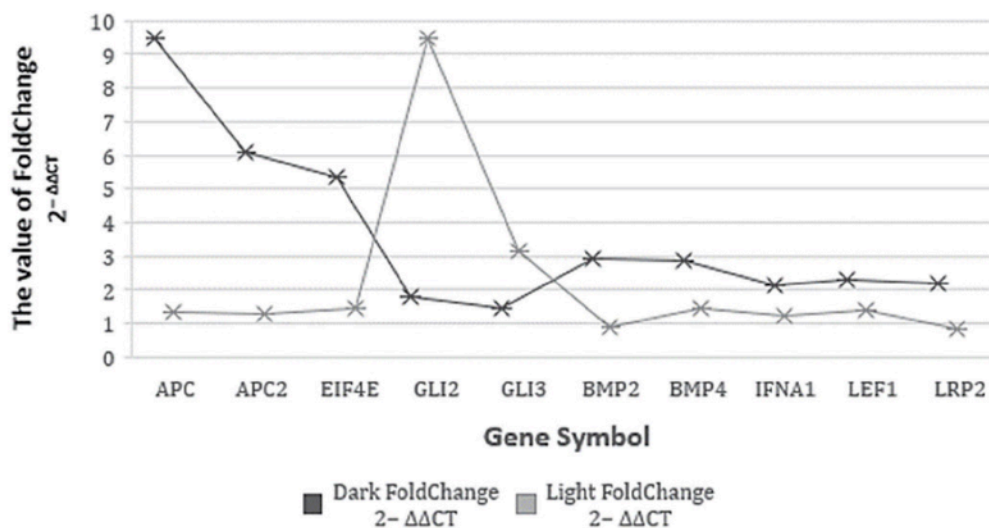
When HT-29 cells were exposed to a specific concentration (of 20  $\mu\text{M}$ ) of ZnPc and a diode laser with a fluence of 5.4  $\text{J}/\text{cm}^2$  was used for light toxicity experiments, the data were analyzed using flow cytometry. HT-29 cells treated with ZnPc showed a higher of cell death opposed to untreated cells ( $**P < 0.01$ ; Figure 2). ZnPc compound led to 53% apoptosis in light, but 22% apoptosis in dark environment. The desired result is minimum death in the dark and maximum death in the light environment. These findings strongly imply the ZnPc's ability to induce apoptosis in HT-29 cells (Figure 2).

### 3. 4. Analyses of Deregulated Genes and Their Related Molecular Mechanism

In cells whose cell viability experiments were finished and whose  $\text{IC}_{50}$  value was 20  $\mu\text{M}$ , the expression levels of about thirty genes were assessed. The  $2^{-\Delta\Delta\text{CT}}$  method was used to calculate the analyses of ten cancer-related genes from the expression levels of thirty genes. The results of the examination of the genes involved in particular cancer pathways showed changes expression levels. The  $2^{-\Delta\Delta\text{CT}}$  explain analyzed ten genes in this pathway and explain their molecular mechanism. To calculate the true fold change at this point, we balance the scales of the genes that are up- and down-regulated using the log base 2 of this value. If not, the scale for upregulation is 2-infinity, while the scale for downregulation is less than 0.5. According to gene expression analyses ten genes including APC, APC2, EIF4E,

**Table 1.** Gene expression in HT-29 cells. Up-regulated genes had a fold change ( $2^{(-\Delta\Delta CT)}$ ) above 2, while down-regulated genes had a  $2^{(-\Delta\Delta CT)}$  below 0.5. Only statistically significant genes are shown. <https://www.genecards.org/>.

Gene Symbol	Gene Name	Dark FoldChange $2^{-\Delta\Delta CT}$	P value $2^{-\Delta\Delta CT}$	Light FoldChange	P value
APC	Adenomatous polyposis coli	9,48	**<0.001	1,33	*<0.05
APC2	Adenomatous polyposis coli	6,08	**<0.001	1,25	*<0.05
EIF4E	Eukaryotic translation initiation factor 4E	5,35	**<0.001	1,46	*<0.05
GLI2	GLI family zinc finger 2	1,76	*<0.05	9,48	**<0.001
GLI3	GLI family zinc finger 3	1,43	*<0.05	3,14	**<0.001
BMP2	Bone morphogenetic protein 2	2,9	*<0.05	0,88	>0.05
BMP4	Bone morphogenetic protein 4	2,87	*<0.05	1,44	*<0.05
IFNA1	human interferon-Alpha1	2,11	>0.05	1,21	*<0.05
LEF1	Lymphoid enhancer binding factor 1	2,32	>0.05	1,39	*<0.05
LRP2	Lipoprotein-receptor-related protein 2	2,18	>0.05	0,84	*<0.05



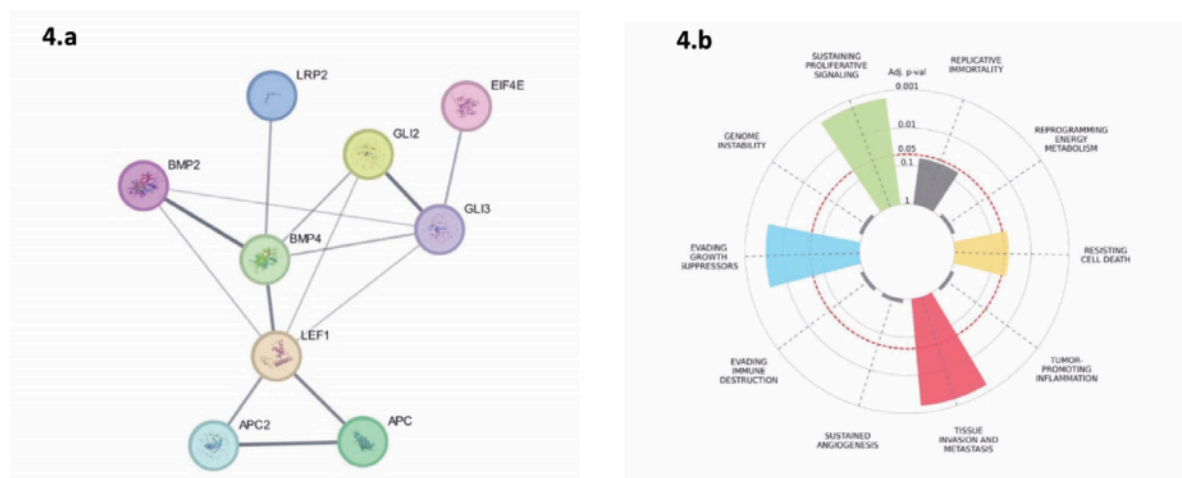
**Figure 3.** Gene expression in HT-29 cells.

GLI2, GLI3, BMP2, BMP4, IFNA1, LEF1, and LRP2 were found statistically significant (Table 1).

Only two genes (GLI2, GLI3) out of ten were significantly ( $p < 0.001$ ) increased compared to the treatment

(light) of the group, while the expression of the rest of the genes was decreased (Figure 3).

PPI network is shown for APC, APC2, EIF4E, GLI2, GLI3, BMP2, BMP4, IFNA1, LEF1, and LRP2 genes in



**Figure 4.** PPI network (4. a) and hallmark cancer analyses (4. b).

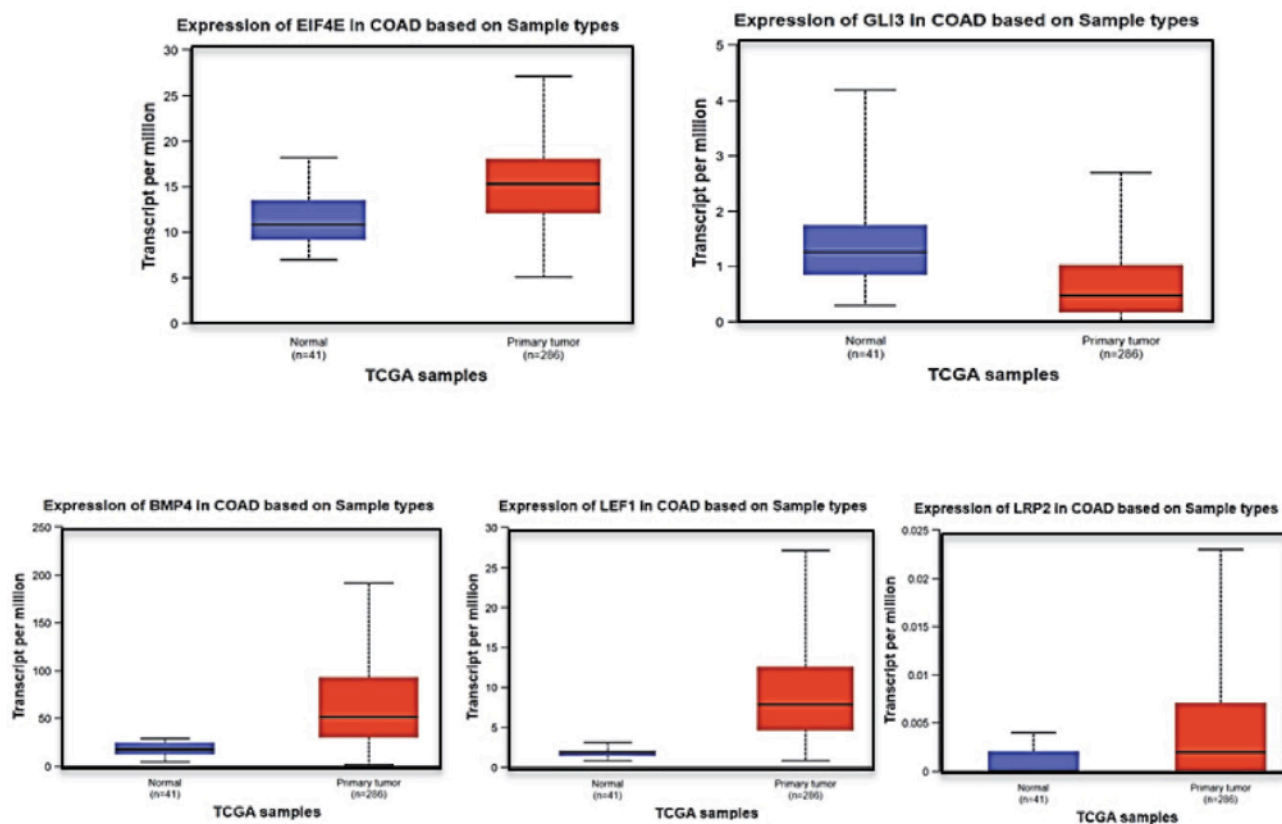


Figure 5. Expression of EIF4E, GLI3, BMP4, LEF1, and LRP2 genes in COAD data using UALCAN tool

Figure 4.a. Enrichment of hallmark cancer analyses shows that our prominent genes have a potential role in sustaining proliferative signaling (LEF1; GLI2; BMP2; BMP4), evading growth suppressors (APC; BMP2; BMP4), tissue invasion and metastasis (APC; LEF1; APC2; BMP2), and resisting cell death (APC; BMP4) molecular mechanisms (Figure 4. b).

In the GO-Biological process analyses using STRING tool, it was determined that all of these 10 genes, which showed expression changes especially with the effect of treatment, were involved in cell differentiation and system development processes. Regarding GO-Molecular process analysis, the beta-catenin binding mechanism (APC; APC2; LEF1; GLI3) emerged in the foreground.

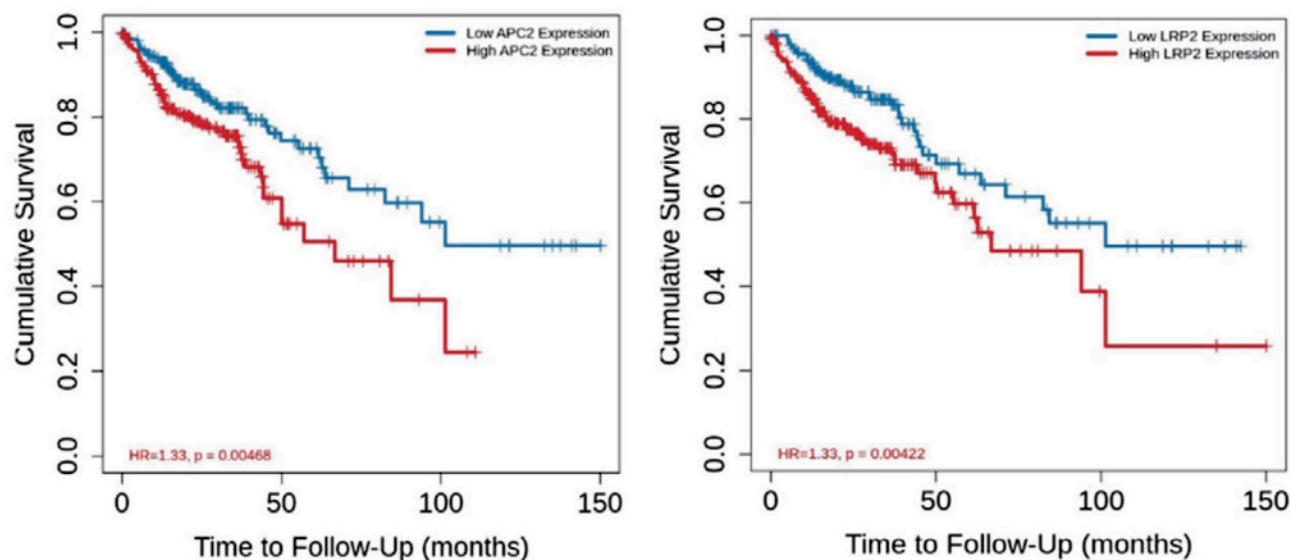


Figure 6. Survival analysis of APC2 and LRP2 genes.

According to the UALCAN tool the genes including EIF4E, BMP4, LEF1, and LRP2 were found increased expression levels and GLI3 was found, the genes including EIF4E, BMP4, LEF1, and LRP2 were found to have increased expression levels, and GLI3 was found to have decreased expression levels in cancer samples compared to normal (Figure 5). In our study expression of EIF4E, BMP4, LEF1, and LRP2 genes decreased and GLI3 increased compared to non-treated (dark) group.

APC and APC2 genes are tumor suppressor genes. In our study, their expression levels were decreased in the treated group compared to the non-treated group. Regarding survival analysis, the APC2 gene has a higher survival rate when its expression level decreases. Moreover, LRP2 gene was also higher survival rate when its expression level decreases (Figure 6).

## 4. Discussion

### 4. 1. In Terms of Cellular Analysis

In certain tumor types, the use of photosensitizers (PS) is favored, and photodynamic therapy (PDT) has long been used as an anti-tumor treatment strategy. Light has long been used effectively to treat disease. It is known that cancer cells use various cell systems to avoid death. They frequently exhibit anti-apoptotic protein overexpression, mutations in proapoptotic proteins, and lysosomal hydrolases that block the initiation of cell death signals.<sup>20</sup> In the flow cytometry experiments, after ZnPc was applied to the HT-29 cells at a concentration of 20  $\mu\text{M}$ , the light power was applied as 5.4 J/cm<sup>2</sup>. Consider analysis results, 52.6% apoptosis was observed in the light environment, while 22.5% apoptosis was observed in the dark. Another study observed 5–20% apoptosis because of PDT applied to HT-29 cells.<sup>21</sup> Thus, we have observed the success of the ZnPc compound, which we use as a photosensitizer, leading to apoptosis.

Jamier et al. investigated the structure-activity relationship of various coumarin derivatives for cytotoxicity against human and mouse carcinoma cell lines (HT29, HepG2, A549, MCF7, OVCAR and CT26). Among the coumarin derivatives, (E)-7-methoxy-4-(3-oxo-3-phenylprop-1-enyl)-2H-chromon-2-one and (E)-7-hydroxy-4-(3-(4-hydroxyphenyl)-3-oxoprop-1-enyl)-2H-chromon-2-one showed the strongest cytotoxic effect on colon cancer cells CT26 (IC<sub>50</sub> = 4.9  $\mu\text{M}$ ) due to their pro-oxidant properties.<sup>22</sup> In our study, according to morphological examination, 20  $\mu\text{M}$  ZnPc significantly suppressed cell growth of HT-29 cell line compared to control groups. According to MTT assay, the effective dose of ZnPc especially for HT-29 cell line was 20  $\mu\text{M}$  (P\*\*\*\* < 0.001).

### 4. 2. In Terms of Molecular Mechanism

APC and APC2 genes act as tumor suppressors. In our study, the expression of these genes showed a decrease

in the response to treatment between the groups compared to the cancer group. In studies, it has been reported that a decrease in the expression of these genes or a tendency to decrease in the treated cancer group leads to an increase in cancer invasion or metastasis formation.<sup>23</sup> However, interestingly, the analysis obtained from TCGA data sets (UALCAN-Gepia2-Oncodb, etc.) shows that low expression of the APC2 gene has a better survival rate. This contradiction needs to be elaborated in detail.

In our prominent genes, there was a tendency for EIF4E expression to decrease with treatment. EIF4E has a vital role in the translation mechanism. A crucial part of the eIF4F trimeric translation initiation complex, eIF4E binds to the 5' cap of eukaryotic mRNAs to control translation.<sup>24</sup> The increasing rate of EIF4E expression was found in many cancer studies. One of these studies was performed by<sup>25</sup> and shows that elevated eIF4E levels in CRC patients have a significant probability of liver metastasis, and eIF4E knockdown prevented CRC cell metastasis by controlling the production of MMP-2, MMP-9, VEGF, and cyclin D1.

In another study was demonstrated that the malignant phenotype of ovarian cancer is largely dependent on the activation of the eIF4E gene, and abnormalities in eIF4E expression are linked to ovarian cancer cell proliferation, migration, invasion, and chemosensitivity to cisplatin.<sup>26</sup> All studies show that downregulation of EIF4E may change the cancer progression.

Among the genes, bone morphogenetic proteins (BMP4), constituting a distinct subset of extracellular multifunctional signaling cytokines, they were first identified as osteogenic factors. They belong to the superfamily of transforming growth factor- $\beta$  (TGF- $\beta$ ).<sup>27</sup> Due to their roles in tumor formation and spread, in addition to embryonic and postnatal development, the identification of BMPs has garnered a lot of attention.<sup>28</sup> Studies indicate that BMPs play a role in both tumor progression and suppression.<sup>29</sup> Although there are some contradictions, especially in colorectal cancer, studies have shown that BMP signaling inhibitors such as BMP type I receptor inhibitor (LDN-193189) induce growth inhibition<sup>30</sup> and apoptosis in cancer cells by decreasing highly expressed BMP4s. Moreover, the administration of this inhibitor to mice also had positive effects on decreasing tumor formation and inducing apoptosis.<sup>31</sup> In our study, BMP4 tended to show a lower expression upon activation of the treatment. This suggests that BMP4 has a positive effect, especially on the apoptotic process.

The Wnt signaling pathway is mediated by a crucial transcription factor called lymphoid enhancer-binding factor 1 (LEF1). LEF1 is a regulator frequently increased in malignancies, such as colonic adenocarcinoma, and strongly linked to tumor aggressiveness. Using shRNA, LEF1 expression was suppressed in a study utilizing caco2 cells. It has been discovered that down-regulation of LEF1 inhibits microstructures linked to motility and malignancy, such as the polymerization of Lamin B1,  $\beta$ -tubulin, and F-actin in caco2 cells. The expression of genes associated

with the epithelial/endothelial-mesenchymal transition (EMT) was decreased by LEF1 inhibition. Additionally, it was reported to induce apoptosis.<sup>32</sup> In vitro knockdown studies in HCT116 cells showed that LEF1 inhibits the proliferation of cancer cells by suppressing the therapeutic efficacy of  $\beta$ -sitosterol.<sup>33</sup> Our results show that LEF1 gene expression tended to decrease expression after treatment activation. Inhibition of LEF1 by this treatment method suggests that it activates apoptotic pathways and also blocks the blocks cell proliferation.

LDL receptor-related protein two is one of the genes that has gained attention since therapy began (LRP2/megalin). The multiliganded endocytic receptor LRP2 is expressed of many tissues, but it is most abundant in absorptive epithelial tissues like the kidney. Although low expression of LRP2 in some cancer types indicates poor survival,<sup>34</sup> with a very low chance of relapse following surgery, a study found that stage II CC cases with significant methylation in LRP2 had different clinical and biological impacts. This gene was shown to be specifically implicated in mechanisms linked to dendritic cell function and B cell immunity, as well as unique characteristics associated with mTORC1 and DNA repair signaling.<sup>35</sup> In our study, a tendency for LRP2 expression to decrease with treatment activation was also found. In addition, survival analysis showed that lower LRP2 expression was associated with better survival in colon cancer.

This study showed that ZnPc is greatly promising in clinical of cancer (especially human colorectal adenocarcinoma).

## 5. Conclusion

These comprehensive findings contribute significantly to our understanding of the nuanced interplay between ZnPc and a 660 nm laser, particularly within the context of HT-29 cells. The research sheds light on the intricate dynamics of energy absorption and subsequent biological responses, emphasizing the potential applications and implications of this light-matter interaction in cellular studies and photodynamic therapy.

## Acknowledgment

We thank Kamil Çınar for his valuable contribution in developing the laser system for this research.

## Conflict of Interest Statement

The authors have no conflicts of interest to declare.

## Ethical Approval

This article does not contain any studies with human or animal subjects.

## Funding

This research received no specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## Availability of Data and Materials

Data is available on request from the authors.

## 6. References

- R.L. Siegel, K.D. Miller, A. Jemal, *CA. Cancer J. Clin.* **2019**, *69*, 7–34. DOI:10.3322/caac.21551
- M. O. Palumbo, P. Kavan, W. H. Miller, L. Panasci Jr., S. Assouline, N. Johnson, V. Cohen, F. Patenaude, M. Pollak, R.T. Jagoe, G. Batist, *Front. Pharmacol.* **2013**, *4*, 57. DOI:10.3389/fphar.2013.00057
- S. Sarkar, N. Cohen, P. Sabhachandani, T. Konry, *Lab. Chip.* **2015**, *15*(23), 4441–50. DOI:10.1039/C5LC00923E
- L. H. Nguyen, A. Goel, D. C. Chung, *Gastroenterology* **2020**, *158*(2), 291–302. DOI:10.1053/j.gastro.2019.08.059
- L. Niu, W. Yang, L. Duan, X. Wang, Y. Li, C. Xu, C. Liu, Y. Zhang, W. Zhou, J. Liu, Q. Zhao, L. Hong, D. Fan, *Mol. Ther. Nucleic Acids* **2020**, *23*, 42–54. DOI:10.1016/j.omtn.2020.10.030
- T. Matsuda, K. Yamashita, H. Hasegawa, T. Oshikiri, M. Hosono, N. Higashino, M. Yamamoto, Y. Matsuda, S. Kanaji, T. Nakamura, S. Suzuki, Y. Sumi, Y. Kakeji, *Ann. Gastroenterol. Surg.* **2018**, *2*(2), 129–136. DOI:10.1002/ags3.12061
- K. Van Der Jeught, H.C. Xu, Y. J. Li, X. Bin Lu, G. Ji, Drug resistance and newtherapies in colorectal cancer, *World J. Gastroenterol.* **2018**, *24*(34), 3834–3848. DOI:10.3748/wjg.v24.i34.3834
- A. Kawczyk-Krupka, A.M. Bugaj, W. Latos, K. Zaremba, K. Wawrzyniec, M. Kucharzewski, A. Sieroń, *Photodiagnosis Photodyn. Ther.* **2016**, *13*, 158–174. DOI:10.1016/j.pdpdt.2015.07.175
- A. F. Dos Santos, D. R. Q. De Almeida, L. F. Terra, M. S. Baptista, L. Labriola, *CA. Cancer J. Clin.* **2011**, *61*(4), 250–81. DOI:10.3322/caac.20114
- C.A. Robertson, D. H. Evans, H. Abrahamse, *J. Photochem. Photobiol. B.* **2009**, *96*(1), 1–8. DOI:10.1016/j.jphotobiol.2009.04.001
- A. Oniszczyk, K. Wojtunik-Kulesza, T. Oniszczyk, K. Kasprzak, *Biomed. Pharmacother.* **2016**, *83*, 912–929. DOI:10.1016/j.biopha.2016.07.058
- R. Baskaran, J. Lee, S. Yang, *Biomater. Res.* **2018**, *22*, 25. DOI:10.1186/s40824-018-0140-z
- N. Q. Khai and T. K. Vu, *Anticancer Agents Med. Chem.* **2024**, *24*(1), 18–29. DOI:10.2174/0118715206272112231102063919
- A. Gok, O. E. Baturhan, U. Salan U, A. R. Özkaya, M. Bulut, *Dyes and Pigments* **2016**, *133*, 311–323. DOI:10.1016/j.dyepig.2016.06.002
- H. Abrahamse, N. N. Houreld, *Int. J. Mol. Sci.* **2019**, *20*(13),

3254. DOI:10.3390/ijms20133254
16. A. G. Yurttas, A. M. Sevim, K. Çınar, G. Y. Atmaca, A. Erdoğan, A. Gül, *Dyes Pigm.* **2022**, *198*, 110012. DOI:10.1016/j.dyepig.2021.110012
17. S. Ozcelik, A. G. Yurttas, M. U. Kahveci, A. M. Sevim, A. Gul, *J. Mol. Struct.* **2023**, *1271*, 134019. DOI:10.1016/j.molstruc.2022.134019
18. P. Mega Tiber, S. Kocyigit Sevin, O. Kilinc, O. Orun, *Gene* **2019**, *692*, 217–222. DOI:10.1016/j.gene.2019.01.015
19. A. G. Yurttas, Z. Okat, T. Elgun, K.U. Cifci, A. M. Sevim, A. Gul, *Photodiagnosis Photodyn. Ther.* **2023**, *42*, 103346. DOI:10.1016/j.pdpdt.2023.103346
20. T. Kirkegaard and M. Jaattela, *Biochim. Biophys. Acta*, **2009**, *1793(4)*, 746–54. DOI:10.1016/j.bbamcr.2008.09.008
21. A. Abedi, F. Tafvizi, P. Jafari and N. Akbari, *Sci Rep.* **2024**, *14(1)*, 3100. DOI:10.1038/s41598-024-53773-y
22. V. Jamier, W. Marut, S. Valente, C. Chereau, S. Chouzenoux, C. Nicco, H. Lemarechal, B. Weill, G. Kirsch, C. Jacob, F. Bateau, *Anticancer Agents Med. Chem.* **2014**, *14(7)*, 963–74. DOI:10.2174/1871520613666131224124445
23. Y. Sun, H. Tian, X. Xu and L. Wang, *Bioengineered* **2020**, *11(1)*, 1027–1033. DOI:10.1080/21655979.2020.1820823
24. M. L. Truitt, C. S. Conn, Z. Shi, X. Pang, T. Tokuyasu, A. M. Coady, Y. Seo, M. Barna and D. Ruggero, *Cell* **2015**, *162(1)*, 59–71. DOI:10.1016/j.cell.2015.05.049
25. T. Xu, Y. Zong, L. Peng, S. Kong, M. Zhou, J. Zou, J. Liu, R. Miao, X. Sun and L. Li, *Oncotargets Ther.* **2016**, *9*, 815–22. DOI:10.2147/OTT.S98330
26. J. Wan, F. Shi, Z. Xu and M. Zhao, *Int. J. Oncol.* **2015**, *47(6)*, 2217–25. DOI:10.3892/ijo.2015.3201
27. X. Guo and X. F. Wang, *Cell Res.* **2009**, *19(1)*, 71–88. DOI:10.1038/cr.2008.302
28. J. C. Hardwick, L. L. Kodach, G. J. Offerhaus and G. R. van den Brink, *Nat. Rev. Cancer* **2008**, *8(10)*, 806–12. DOI:10.1038/nrc2467
29. S. Ehata, Y. Yokoyama, K. Takahashi and K. Miyazono, *Pathol. Int.* **2013**, *63(6)*, 287–96. DOI:10.1111/pin.12067
30. S. Nunomura, H. Ota, T. Irisawa, K. Endo and Y. Morita, *Appl. Phys. Express* **2023**, *16*, 061004. DOI:10.35848/1882-0786/acdc82
31. Y. Yokoyama, T. Watanabe, Y. Tamura, Y. Hashizume, K. Miyazono, S. Ehata, *Cancer Res.* **2017**, *77(15)*, 4026–4038. DOI:10.1158/0008-5472.CAN-17-0112
32. L. Xiao, C. Zhang, X. Li, C. Jia, L. Chen, Y. Yuan, Q. Gao, Z. Lu, Y. Feng, R. Zhao, X. Zhao, S. Cheng, Z. Shu, J. Xu, W. Duan, G. Nie, Y. Hou, *Int. J. Mol. Sci.* **2021**, *22(19)*, 10870. DOI:10.3390/ijms221910870
33. S. Gu, F. Liu, X. Xie, M. Ding, Z. Wang, X. Xing, T. Xiao, X. Sun, *Cell. Signal.* **2023**, *104*, 110585. DOI:10.1016/j.cellsig.2022.110585
34. M. Q. Rasmussen, G. Tindbæk, M. M. Nielsen, C. Merrild, T. Steiniche, J. S. Pedersen, S. K. Moestrup, S. E. Degn, M. Madsen, *Cancers (Basel)* **2023**, *15(6)*, 1830. DOI:10.3390/cancers15061830
35. B. Tournier, R. Aucagne, C. Truntzer, C. Fournier, F. Ghiringhelli, C. Chapusot, L. Martin, A. M. Bouvier, S. Manfredi, V. Jooste, M. B. Callanan, C. Lepage, *Cancers* **2023**, *15(1)*, 158. DOI:10.3390/cancers15010158

## Povzetek

Fotodinamična terapija (FDT) je način zdravljenja raka. Singletni kisik nastane kot posledica fotokemične reakcije med svetlobo, fotosenzibilizatorjem in molekularnim kisikom, ki uničuje celice. Pri raku debelega črevesa, za katerim na svetu boleha 1,23 milijona ljudi, je pogosto potrebna operacija, vendar je stopnja ponovitve bolezni in metastaz zelo visoka. FDT zato predstavlja alternativno pri zdravljenju raka debelega črevesa. V tej študiji so bili za oceno viabilnosti celic uporabljeni testi MTT, fotosenzibilizator cink(II) ftalocianin (ZnPc) pa je bil uporabljen na celični liniji adenokarcinoma debelega črevesa (HT-29) z namenom razjasnitve poti razvoja raka s pretočno citometrijo in q-PCR. Rezultati so pokazali, da je FDT z ZnPc znatno zmanjšala viabilnost celic HT-29 in povzročila apoptozo 53 % celic. Glede na rezultate q-PCR so bile vrednosti CT desetih od tridesetih genov signifikantne, zato je bila ovrednotena njihova povezava z rakom.



Except when otherwise noted, articles in this journal are published under the terms and conditions of the Creative Commons Attribution 4.0 International License