ANTICANCER RESEARCH *37*: 6251-6258 (2017)
doi:10.21873/anticanres.12076

# The Anticancer Effects of Radachlorin-mediated Photodynamic Therapy in the Human Endometrial Adenocarcinoma Cell Line HEC-1-A

SU-MI KIM[1], YUN-HEE RHEE[2] and JONG-SOO KIM[1]

[1]*Department of Obstetrics and Gynecology, College of Medicine, and*
[2]*Beckman Laser Institute Korea, Dankook University, Cheonan, Republic of Korea*

**Abstract.** *Aim: We investigated the effect of photodynamic therapy (PDT) using radachlorin on invasion, vascular formation and apoptosis by targeting epidermal growth factor receptor (EGFR)/vascular endothelial growth factor receptor 2 (VEGFR2) signaling pathways in the HEC-1-A endometrial adenocarcinoma cell line. Materials and Methods: To investigate the apoptotic pathway, we performed the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay, and western blot analysis. We also evaluated the effects of PDT on tubular capillary formation in and invasion by HEC-1-A cells with a tube formation assay, invasion assay, prostaglandin E2 (PGE2) assay, and western blot analysis. Results: PDT had anticancer effects on HEC-1-A through activation of the intrinsic pathway of apoptosis via caspase-9 and poly-(ADP-ribose) polymerase (PARP). PDT also inhibited tubular capillary formation in and invasion by HEC-1-A under VEGF pretreatment, that resulted from down-regulation of VEGFR2, EGFR, Ras homolog gene family/ member A (RhoA) and PGE2. These results are indicative of the specificity of radachlorin-mediated PDT to VEGF. Conclusion: The major advantage of radachlorin-mediated PDT is its selectivity for cancer tissue while maintaining adjacent normal endometrial tissue. Therefore, radachlorin-mediated PDT might offer high anticancer efficacy for endometrial adenocarcinoma and an especially useful modality for preserving fertility.*

*Correspondence to:* Professor Jong-Soo Kim, Ph.D., Department of Obstetrics & Gynecology, College Of Medicine, Dankook University, 359 Manhyangro, Dongnam-gu, Cheonan, Chungcheongnam-do, 330-715, Republic of Korea. Tel: +82 415506159, Fax: +82 415563878, e-mail: soo8541@hanmail.net

*Key Words:* Radachlorin, photodynamic therapy, endometrial adenocarcinoma cell line, HEL-1-A, EGFR, apoptosis.

Endometrial cancer is the third most common malignancy in Korea and the most common gynecological cancer in the United States (1, 2). The burden of endometrial cancer has increased with an annual increase in incidence and mortality. Approximately 80% of endometrial cancers are diagnosed at an early stage (3, 4) and 3-14% of all cases are diagnosed before 40 years of age (5). Conservative therapy has emerged as an alternative to surgery in the management of young patients with early-stage endometrial cancer who wish to retain their fertility. Hormonal agents, such as progestin, and gonadotropin-releasing hormone analogs are often used in such cases (6). However, maintenance treatment with hormonal agents precludes pregnancy; moreover, the therapeutic effect appears to be inadequate. Therefore, development of new treatment modalities for endometrial cancer is required for women who wish to preserve their fertility.

Epidermal growth factor receptor (EGFR) and vascular endothelial growth factor receptor 2 (VEGFR2) are tyrosine kinase receptors that play a key role in cancer development and progression. Activation of EGFR and VEGFR2 triggers the phosphorylation of multiple proteins, including those of the phosphatidylinositol-3-kinase/serine/threonine kinase (PI3K/AKT) and mitogen-activated protein kinase (MAPK) pathways which are involved in cell survival, apoptosis and angiogenesis (7, 8). Overexpression of EGFR and VEGFR2 is frequently observed in many cancer types (9, 10). Abnormal activation of EGFR and VEGFR2 correlates with poor prognosis and chemoresistance (11, 12). Because of potential interactions and their well-established role in cancer growth and angiogenesis, inhibition of both EGFR and VEGFR2 signaling pathways might improve the outcome of patients with endometrial cancer (13).

Photodynamic therapy (PDT) is becoming widely accepted as a potential treatment for cancer and some non-malignant diseases (14). Some studies indicated the potential therapeutic role of PDT in patient with early-stage endometrial cancer (15, 16). PDT involves the use of a

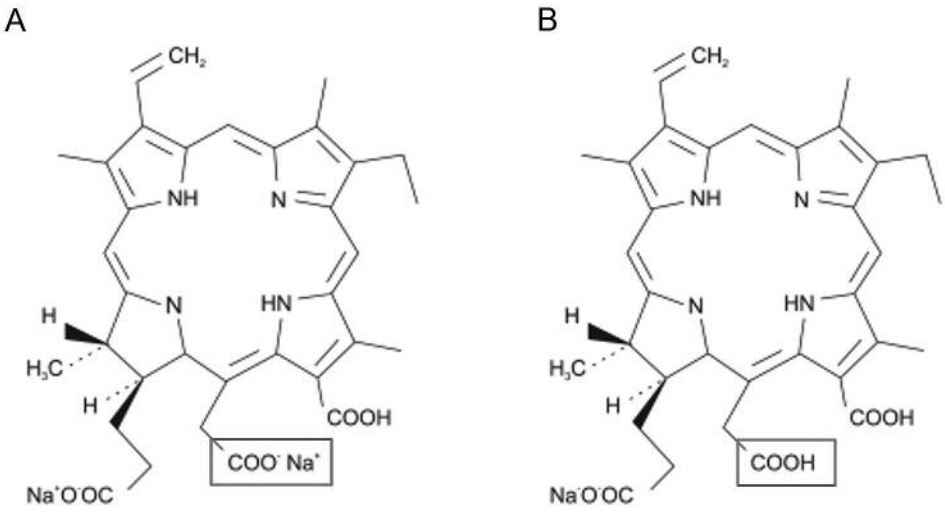


Figure 1. *Structure of radachlorin®. A: Major component. sodium chlorine $e_6$; B: one of the minor components, sodium chlorine $e_6$.*

compound called a photosensitizer that can be excited by a specific wavelength of light. The activated photosensitizer then interacts with molecular oxygen to produce cytotoxic singlet oxygen and other reactive oxygen species that destroy target cells (17). Radachlorin, a mixture of the sodium salts of chlorine e6, chlorine p6 and purpurin 18, is a promising second-generation photosensitizer (Figure 1). Radachlorin has better light penetration of target tissue, as compared to first-generation photosensitizers (18). The anticancer effects of PDT using radachlorin in some *in vitro* and *in vivo* cancer models have been reported (19-22); however, the potential of radachlorin-mediated PDT for the treatment of endometrial adenocarcinoma remains unclear.

In the present study, we investigated the effect of radachlorin-mediated PDT on invasion, vascular formation and apoptosis by targeting EGFR/VEGFR2 signaling pathways in the HEC-1-A endometrial adenocarcinoma cell line.

## Materials and Methods

*Cell lines and cell culture*. The human endometrial adenocarcinoma cell line HEC-1-A was purchased from the American Type Culture Collection (Manassas, VA, USA). HEC-1-A was maintained in McCoy's 5A medium (Life Technologies, Grand Island, NY, USA) containing 10% fetal bovine serum and 1% streptomycin-penicillin (Corning, Carlsbad, CA, USA).

*Photosensitizer and light source*. Radachlorin (RADA-PHARMA Co, Ltd., Moscow, Russia) was dissolved in dimethylsulfoxide (DMSO) to obtain 1 mM stock solution, which was stable in solution at 0±4˚C in the dark. Working drug solutions (0.39-200 μg/ml) were prepared by diluting the stock solution with serum-free medium. A diode laser device (WonTech, Korea; power output 50 mW) was the source of monochromatic red light (660 nm) for PDT used in this study.

*Photodynamic treatment and observation of cell morphology*. HEC-1-A cells were seeded at a density of $5\times10^4$ cells per well in 96-well flat-bottomed micro titer plates (SPL Life Science, Daejeon, Korea). After 24 h, cells were incubated with different concentrations of radachlorin for 4 h in the dark. After removing the drug and washing with phosphate-buffered saline (PBS), the treated cells was irradiated using a light-emitting diode at 25 J/cm$^2$. All irradiations were performed at room temperature (25˚C). Controls for each experiment were cells exposed to PDT light (at each light dose) without radachlorin. Cells were observed at 24 and 48 h under an inverted microscope (BX51; Olympus, Miami, FL, USA.). Cytototoxicity by PDT results were expressed as the percentage of treated cell viability in comparison to controls. All experiments were repeated three times.

*Assessment of cell viability: 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay*. Cell survival was evaluated by MTT assay. In brief, culture medium was removed, and control/treated cells were incubated in medium with 0.5 mg/ml MTT solution (Sigma-Aldrich Corporation, St. Louis, MO, USA) for 3 h at 37˚C to allow MTT metabolism. The resulting formazan crystals were dissolved with 100 mol/l of DMSO (Life technologies, Grand Island, NY, USA) and the absorbance was measured at 540 nm using an enzyme-linked immunosorbent assay (ELISA) plate reader (Tecan, Austria).

*Terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labeling (TUNEL) assay*. TUNEL assay was performed by TdT-FragELTM DNA fragmentation Detection Kit (Oncogene, Boston, MA, USA) according to the manufacturer's instructions. Briefly, cells were seeded in a chamber slide and incubated with 25 μM of radachlorin described previously. After PDT, cells were incubated for 24 and 48 h and were fixed using 2% formaldehyde. After fixation, cells were permeabilized with 2.5% triton X-100 for 10min and washed with PBS. TdT-Annexin V labeling reaction mixture in equilibrium buffer was added to specimens and incubation

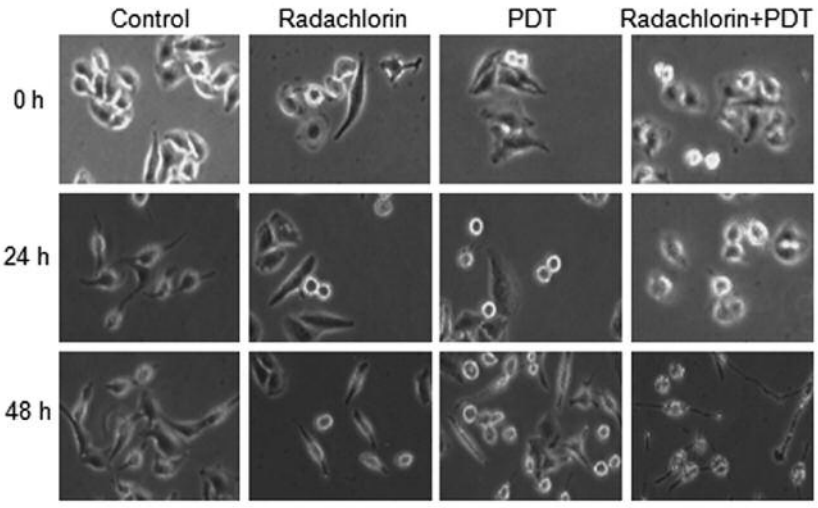


Figure 2. *Morphological observation under an inverted microscope: the control cells grew adherently, showing clear contour. However, photodynamic therapy (PDT)-treated cells had altered morphology, showing condensed cytoplasm or floating behavior.*

performed for 2 h. After incubation, specimens were counterstained with 20 μg/ml of propidium iodide (PI) and annexin V-positive cells were observed by fluorescence microscope.

*Tube formation assay*. Plates were coated with matrigel (BD Biosciences, Bedford, MA, USA) by incubation at 37˚C for 30 min. Cells ($3\times10^4$) were seeded with 50 ng/ml of recombinant human VEGF (Peptrotech, Rocky Hill, NJ, USA) onto the 24-well matrigel-coated plates. After 8 h incubation, cells were fixed with 2% formaldehyde and were stained with 2% crystal violet. Tube formation was observed under microscopy (BX51; Olympus) and total tube area was measured using Image J program (NIH, Bethesda, MD, USA).

*Invasion assay*. The invasive ability of HEC-1-A cells was measured by the Boyden chamber invasion assay. Matrigel was applied to the top side of 8-μm pore polycarbonate filters. In a Boyden chamber consisting of two chambers. A total of 30 μl of medium from HEC-1-A cells cultured for 24 h was applied in the lower chamber, and in the upper chamber, HEC-1-A cells were seeded at a density of $1\times10^5$ cells with or without 50 ng/ml of recombinant human VEGF (Peptrotech, Rocky Hill, NJ, USA). The chamber underwent PDT and was incubated for 8 h at 37˚C. At the end of incubation, the cells in the upper surface of the membrane were carefully removed with a cotton swab and cells that had invaded across the matrigel to the lower surface of the membrane were fixed with methanol and stained with 5% Giemsa solution. The invading cells on the lower surface of the membrane filter were counted with a light microscope. The data are presented as the average number of cells attached to the bottom surface from randomly chosen fields. Each experiment was carried out in triplicate.

*Prostaglandin $E_2$ ($PGE_2$) assay*. The expression level of PGE2 in cell culture medium was determined using an ELISA. Cell culture medium was collected from tube formation assay supernatant which was concentrated using centricon (Millipore, Temecula, CA, USA). The ELISA kits were purchased from R&D Systems (Minneapolis, MN, USA) and assays were performed according to the manufacturer's instructions and repeated in triplicate.

*Western blot analysis*. HEC-1-A cells were seeded at a density of $1\times10^5$ cells per well onto 6 wells with or without 50 ng/ml of recombinant human VEGF (Peptrotech, Rocky Hill, NJ, USA) before PDT. For western blot analysis, cell lysates were prepared with lysis buffer [50 mM Tris-HCl, (pH 7.5), 1 mM ethylenediaminetetra-acetic acid, 150 mM NaCl, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 10 μg/ml pepstatin A, 1 mM phenylmethylsulfonyl fluoride, 1 mM sodium fluoride, 10 mM iodoacetamide, 1 mM sodium orthovanadate] for 1 h on ice then centrifuged twice at 10,000 × *g* for 15 min. Equal amounts of lysates (50 μg) were boiled in sodium dodecyl sulfate sample buffer and subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). After transfer, immuno-active products were detected by ECL system (GE, Piscataway, NJ, USA). Monoclonal antibodies to poly-(ADP-ribose) polymerase (PARP), caspase-9, VEGFR2, Ras homolog gene family/ member A (RhoA), and EGFR were obtained from Cell Signaling (Beverly, MA, USA). Mouse monoclonal antibody to β-actin (Sigma-Aldrich Corporation, St. Louis, MO, USA) was used as a loading control.

*Statistical analysis*. Results are expressed as the mean±S.D. The statistical significance of differences between the groups was determined by applying Student's *t*-test. Values of $p<0.05$ were considered statistically significant.

## Results

*The morphological observation of HEC-1-A cells after PDT*. In order to investigate whether radachlorin-mediated PDT induced cell death, HEC-1-A cells were observed under an inverted microscope 24 and 48 h after treatment. As shown in

Figure 2, the control cells grew adherently, showing a clear contour. However, PDT-treated cells had altered morphology, showing condensed cytoplasm or floating growth pattern within 24 h.

*Viability of HEC-1-A cells was reduced with PDT.* The effect of different concentrations of radachlorin with PDT on HEC-1-A cell viability was determined by MTT assay. Figure 3 shows that the viability of cells treated with radachlorin and PDT decreased on treatment with 12.5 to 200 μM radachilorin. After 24 h incubation, the half-maximal inhibitory concentration ($IC_{50}$) for radachlorin was 55.4 μM, and after 48 h incubation, this was reduced to 20 μM. Based on these results, 25 μM of radachlorin and 48-h incubation time were selected for further studies.

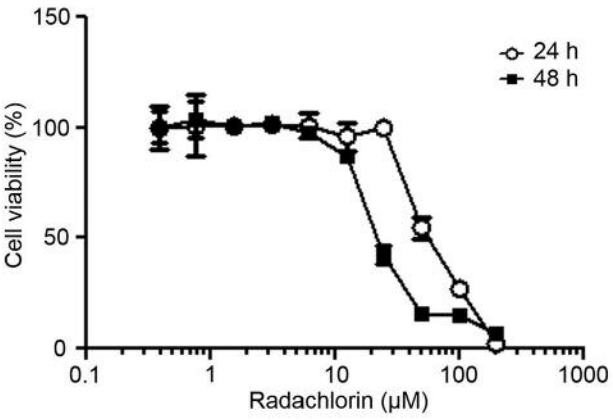


Figure 3. *3-(4,5-Dimethylthizol-2-yl)-2,5-diphenyltetrazolium bromide assay. Cell viability was reduced by increasing concentrations of radachlorin. Based on the results, 25 μM of radachlorin and 48 h incubation time were selected for further studies.*

*PDT induced apoptosis of HEC-1-A cells.* TUNEL assay determines nuclear fragmentation by using TdT to transfer fluorescein-12-d UPT to free 3'-OH of cleaved DNA (16). Apoptotic cells produce fragmented nuclear DNA, which is stained brightly using annexin-V and PI labeling. From Figure 4, it can be seen that radachlorin-mediated PDT led to a significantly increased number of TUNEL-positive cells, implying that apoptosis was enhanced in comparison to controls. The effect of apoptosis was significantly stronger at 48 h incubation, compared to 24 h incubation.

*Radachlorin-mediated PDT augmented the apoptosis signaling pathways.* Caspases are cysteine proteases that are activated during the apoptotic signaling pathways. PARP belongs to a family of proteins mainly involving DNA repair and programmed cell death. Expression of PARP and caspase-9 were analyzed to study the involvement of the apoptosis signaling pathways. Expression of PARP and caspase-9 were significantly increased in VEGF-pretreated HEC-1-A cells treated with PDT in comparison to cells not pretreated with VEGF, as shown in Figure 5.

*PDT reduced tubular formation of HEC-1-A cells.* Tubular formation in endometrial cells has an important role in angiogenesis. VEGF induction of VEGFR results in tubular capillary formation in endometrial cells, which is related to cell invasion. The inhibitory effects of PDT on tubular formation in HEC-1-A cells were assessed using *in vitro* capillary-like structure (tube) formation assay. Cells treated with PDT alone showed a decrease in total tube area ($p$=0.0029) compared to cells treated with VEGF alone (Figure 6). Interestingly, PDT treatment of VEGF-pretreated cells led to stronger inhibition of tubular formation in comparison to PDT of cells not pretreated with VEGF ($p$=0.0036) (Figure 6).

*PDT suppressed invasion by HEC-1-A cells.* Cancer cell invasion is an important step in metastasis. We investigated the inhibitory effect of PDT on HEC-1-A invasiveness using a Boyden chamber. PDT blocked invasion by HEC-1-A cells, as compared to controls ($p$=0.0084) (Figure 7). Likewise, HEC-1-A invasiveness was markedly reduced by PDT in VEGF pretreated cells ($p$=0.0087).

*PDT reduced PGE2 production of HEC-1-A cells.* PGE2 is a pro-inflammatory, mitogenic, and pro-angiogenic molecule. As shown in Figure 8, PGE2 levels decreased significantly in PDT-treated HEC-1-A cells ($p$=0.0002). Likewise, the inhibitory effect of PDT was stronger in VEGF-pretreated cells ($p$=0.0008).

*Expression of EGFR, VEGFR2, and RhoA were reduced with radachlorin-mediated PDT.* Expression of EGFR, VEGFR2 and RhoA were analyzed in order to investigate the anti-angiogenic effects of PDT. As shown in Figure 9, expression of VEGFR2, EGFR and RhoA were reduced by PDT under VEGF pretreatment after 48 h incubation.

## Discussion

PDT is an evolving modality for treating malignant neoplasms through its potential to eliminate microscopic cancer cells. Among gynecologic cancers, PDT is reportedly effective for cervical, vaginal, vulvar, and early endometrial cancers (15, 16, 23-27). PDT is less damaging to normal surrounding tissue, indicating enhanced tumor selectivity. Mirzaei *et al.* reported that HepG2, a hepatocellular carcinoma cell line, was more sensitive to the lethal effects of radachlorin- PDT than HFLF-PI4, a normal liver cell line (18). Therefore, PDT is a promising alternative for selective therapy of tumor cells.

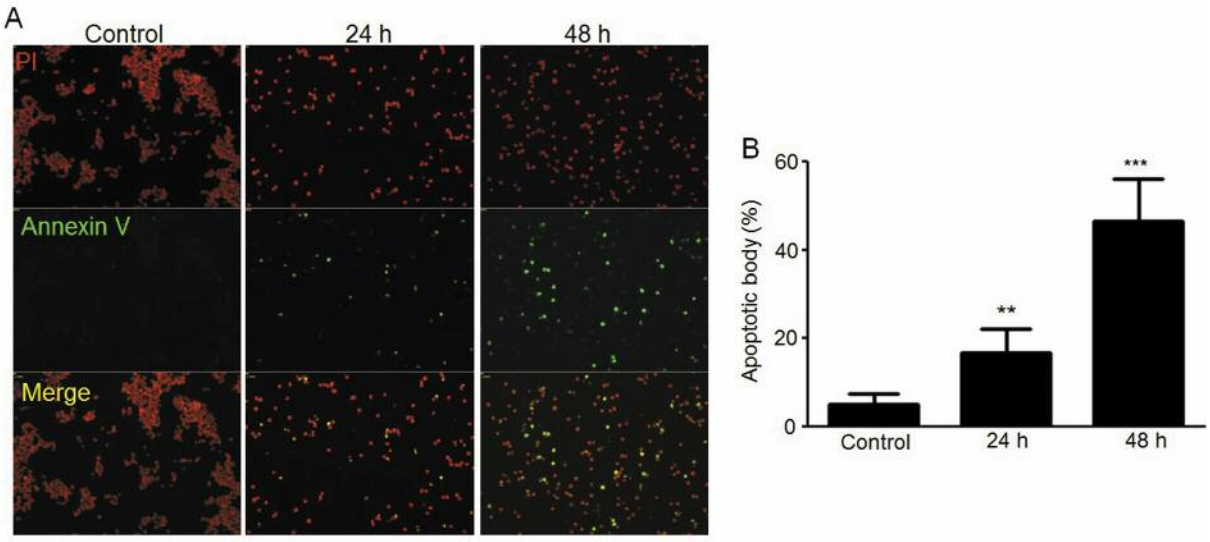


Figure 4. *Terminal deoxynucleotidyl transferase dUTP nick end-labeling (TUNEL) assay. A: Cells were labelled with annexin-V and propidium iodide (PI) to study apoptosis. Radachlorin-mediated photodynamic therapy (PDT) led to a higher proportion of TUNEL-positive cells, implying that apoptosis was enhanced in comparison to controls. The effect on apoptosis was significantly stronger with 48-h incubation than 24-h incubation. B: The histogram shows that the number of apoptotic cells increased significantly with radachlorin-mediated PDT. Significantly different from the control at **p=0.0026 and ***p<0.0001.*

The objective of this study was to evaluate the efficacy of radachlorin-mediated PDT on HEC-1-A, a human endometrial adenocarcinoma cell line. We also investigated the signaling pathways involved in anticancer effects of radachlorin-mediated PDT.

Our study suggests that radachlorin-mediated PDT significantly inhibited cell invasion and vascular formation, and enhanced apoptosis of HEC-1-A cells.

MTT and TUNEL assay showed that radachlorin-mediated PDT led to a higher number of apoptotic cells. Caspase- 9 is implicated as an initiator caspase, triggering the intrinsic pathway of apoptosis. Once activated, it leads to cleavage of various cytoplasmic or nuclear substrates, including PARP. Our western blot assay showed up-regulation of caspase-9 and PARP on treatment of HEC-1-A cells with radachlorin-mediated PDT. These results indicate that the intrinsic pathway of apoptosis *via* caspase-9 and PARP through inhibition of EGFR/VEGFR2 is the main pathway by which apoptosis was induced by PDT in HEC-1-A cells.

Cell migration and invasion are landmark events that transform a locally growing tumor into a systemic, metastatic disease. Cancer invasion is a cell- and tissue-driven process by which the physical, cellular and molecular determinants adapt and react throughout disease progression (28). Angiogenesis, the formation of new blood vessels from pre-existing vessels, is an essential process in malignant tumor invasion (29). Radachlorin-mediated PDT significantly

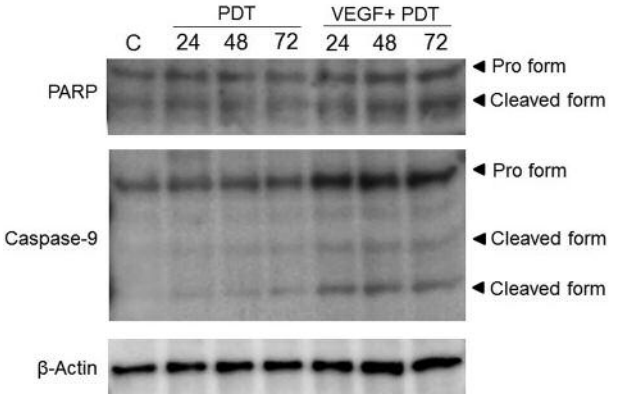


Figure 5. *Western blot analysis of poly-(ADP-ribose) polymerase (PARP) and caspase-9. Expression of apoptosis pathway-related proteins PARP and caspase-9 was significantly increased in vascular endothelial growth factor (VEGF)- pretreated HEC-1-A cells treated with photodynamic therapy (PDT) in comparison to cells not pretreated with VEGF. C: Untreated control cells.*

reduced invasion and vascular formation by HEC-1-A-cells. Known regulatory mechanisms of angiogenesis include angiogenic induction with VEGF; however, recent studies indicate that the angiogenic response is more complex and may involve many factors (30). Among the many angiogenic

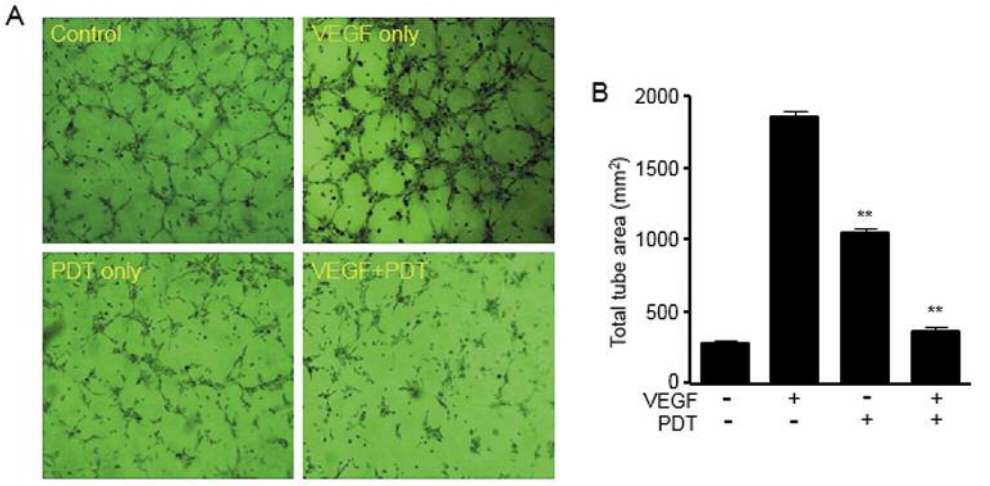


Figure 6. *Tube formation assay. A: Measuring the area of capillary-like structures under microscopy (×20) shows inhibitory effects on tubular formation by photodynamic therapy (PDT). B: PDT led to a decrease in total tube area, as compared to control. Interestingly, PDT treatment in vascular endothelial growth factor (VEGF)-pretreated cells showed stronger inhibition of tubular formation in comparison to cells not pretreated with VEGF.*

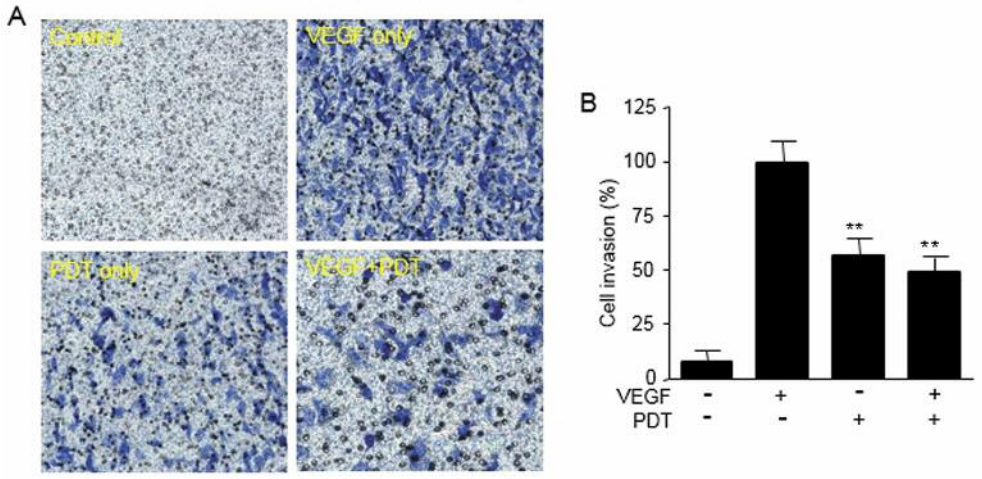


Figure 7. *Invasion assay. A: Invasive cells on the lower surface of the membrane filter as seen using a light microscope (×40). B: Photodynamic therapy (PDT) significantly blocked invasion by HEC-1-A cells compared to controls. HEC-1-A invasiveness was markedly reduced by PDT in vascular endothelial growth factor (VEGF)-pretreated cells.*

factors, VEGFR2, EGFR, PGE2 and RhoA were selected in our study. PGE2, the predominant prostaglandin in solid tumors, is synthesized in cancer epithelial, blood vessel endothelial, and immune cells (31). PGE2 exerts its effects in an autocrine and paracrine fashion and affects cancer cell growth, survival, and migration (32). Available evidence suggests that PGE2 might induce proangiogenic factors, such as VEGF, which initiate angiogenesis of endothelial cells. Our results showed that radachlorin-mediated PDT suppressed PGE2 production of HEC-1-A cells. The VEGFR/EGFR signaling pathway is critical for endothelial cell proliferation, migration, cell survival and for the induction of vascular permeability (33).

One of the small GTPases, RhoA, controls a diverse array of cellular processes, including cytoskeletal dynamics, cell polarity, membrane transport, and gene expression (34). Rho signaling is reportedly essential for VEGF dependent *in vivo* angiogenesis and *in vitro* capillary formation (35-37). With

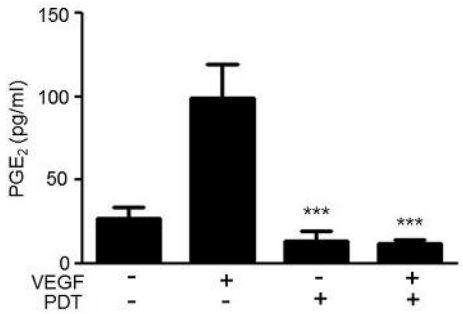


Figure 8. *Prostaglandin E2 (PGE2) assay showed $PGE_2$ expression significantly decreased in photodynamic therapy (PDT)-treated HEC-1-A cells. The inhibitory effect of PDT was stronger in cells treated with vascular endothelial growth factor (VEGF).*

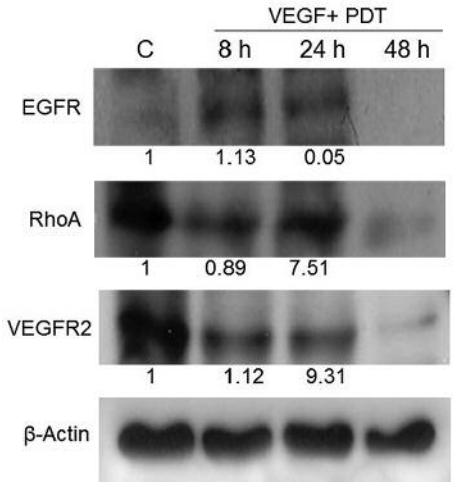


Figure 9. *Western blot analysis of epidermal growth factor receptor (EGFR), Ras homolog gene family/ member A (RhoA), and vascular endothelial growth factor receptor-2 (VEGFR2). Expression of VEGFR2, EGFR and RhoA were inhibited by PDT after 48 h incubation under pretreatment with VEGF. The expression folds comparing untreated cells (C) were measured using Image J, and normalized with β-actin.*

certain angiogenic signals including VEGF, RhoA is converted from its inactive (GDP-bound) to active form (GTP-bound) *via* catalysis from certain Rho guanine nucleotide exchange factors (38). Brad *et al*. suggested that Rho inhibition blocks VEGF-driven vessel formation (39). In our study, results from western blot analysis showed down-regulation of VEGFR2, EGFR and RhoA, which suggests that radachlorin-mediated PDT inhibits multiple pathways involved in cancer invasion and vascular formation. Interestingly, tube formation and invasion assay results showed strong anticancer effects on cells pretreated with VEGF, indicative of the specificity of radachlorin-mediated PDT to VEGF-expressing cells.

Therefore, different signaling pathways were modulated in radachlorin-mediated PDT. Firstly, the angiogenic pathway was suppressed by inhibition of VEGFR2, EGFR, PGE2, and RhoA. Secondly, intrinsic apoptosis was activated *via* caspase- 9 and PARP.

These findings could be of importance in developing PDT as a fertility-preserving therapy for patients with gynecolgical cancer.

## Conflicts of Interest

The Authors declare that they have no conflict of interest in regard to this study.

## References

1 Lim MC, Moon EK, Shin A, Jung KW, Won YJ, Seo SS, Kang S, Kim JW, Kim JY and Park SY: Incidence of cervical, endometrial, and ovarian cancer in Korea 1999-2010. J Gynecol Oncol *24*: 298-302, 2013.

2 Siegel R, Naishadham D and Jemal A: Cancer statistics 2013. CA Cancer J Clin *63*: 11-30, 2013.

3 Rose PG: Endometrial carcinoma. N Engl J Med *335*: 640-649, 1996.

4 Amant F, Moerman P, Neven P, Timmerman D, Van Limbergen E and Vergote I: Endometrial cancer. Lancet *366*: 491-505, 2005.

5 Akimasa T, Fuminori K, Akiyoshi Y, Akie T, Nobuyuki K, Kentaro T and Takashi M: Metformin impairs growth of endometrial cancer cells *via* cell-cycle arrest and concomitant autophagy and apoptosis. Cancer Cell Int *14*: 53, 2014.

6 Choi MC, Jung SG, Park H, Cho YH, Lee C and Kim SJ: Fertility preservation *via* photodynamic therapy in young patients with early-stage uterine endometrial cancer: a long-term follow-up study. Int J Gynecol Cancer *23*: 698-704, 2013.

7 Gerber HP, McMurtrey A, Kowalski J, Yan M, Keyt BA, Dixit V and Ferrara N: Vascular endothelial growth factor regulates endothelial cell survival through the phosphatidylinositol 3'-kinase/AKT signal transduction pathway. Requirement for Flk-1/KDR activation. J Biol Chem *273*: 30336-30343, 1998.

8 Takahashi T, Ueno H and Shibuya M: VEGF activates protein kinase C-dependent, but RAS-independent RAF-MEK-MAP kinase pathway for DNA synthesis in primary endothelial cells. Oncogene *18*: 2221-2230, 1999.

9 Yarden Y: The EGFR family and its ligands in human cancer. signalling mechanisms and therapeutic opportunities. Eur J Cancer *37(Suppl 4)*: S3-8, 2001.

10 Ferrara N, Gerber HP and LeCouter J: The biology of VEGF and its receptors. Nat Med *9*: 669-675, 2003.

11 Nicholson RI, Gee JM and Harper ME: EGFR and cancer prognosis. Eur J Cancer *37(Suppl 4)*: S9-15, 2001.

12 Yang F, Tang X, Riquelme E, Behrens C, Nilsson MB, Giri U, Varella-Garcia M, Byers LA, Lin HY, Wang J, Raso MG, Girard L, Coombes K, Lee JJ, Herbst RS, Minna JD, Heymach JV and Wistuba II: Increased *VEGFR2* gene copy is associated with chemoresistance and shorter survival in patients with non-small-cell lung carcinoma who receive adjuvant chemotherapy. Cancer Res *71*: 5512-5521, 2001.

13 Pal HC, Sharma S, Strickland LR, Agarwal J, Athar M, Elmets CA and Afaq F: Delphinidin reduces cell proliferation and induces apoptosis of non-small-cell lung cancer cells by targeting EGFR/VEGFR2 signaling pathways. PLoS One *8*: e77270, 2013.
14 Hopper C: Photodynamic therapy: a clinical reality in the treatment of cancer. Lancet Oncol *1*: 212-219, 2000.
15 Ehrlich CE, Young PC, Stehman FB, Sutton GP and Alford WM: Steroid receptors and clinical outcome in patients with adenocarcinoma of the endometrium. Am J Obstet Gynecol *158*: 796-807, 1988.
16 Qiang YG, Zhang XP, Li J and Huang Z: Photodynamic therapy for malignant and non-malignant diseases: clinical investigation and application. Chin Med J (Engl) *119*: 845-857, 2006.
17 Robertson CA, Evans DH and Abrahamse H: Photodynamic therapy (PDT): a short review on cellular mechanisms and cancer research applications for PDT. J Photochem Photobiol B *96*: 1-8, 2009.
18 Mirzaei H, Djavid GE, Hadizadeh M, Jahanshiri-Moghadam M and Hajian P: The efficacy of Radachlorin-mediated photodynamic therapy in human hepatocellular carcinoma cells. J Photochem Photobiol B *142*: 86-91, 2015.
19 Kochneva EV, Filonenko EV, Vakulovskaya EG, Scherbakova EG, Seliverstov OV, Markichev NA and Reshetnickov AV: Photosensitizer Radachlorin®: Skin cancer PDT phase II clinical trials. Photodiagnosis Photodyn Ther *7*: 258-267, 2010.
20 Bae SM, Kim YW, Lee JM, Namkoong SE, Han SJ, Kim JK, Lee CH, Chun HJ, Jin HS and Ahn WS: Photodynamic effects of Radachlorin on cervical cancer cells. Cancer Res Treat *36*: 389-394, 2004.
21 Filonenko EV, Sokolov VV, Chissov VI, Lukyanets EA and Vorozhtsov GN: Photodynamic therapy of early esophageal cancer. Photodiagnosis Photodyn Ther *5*: 187-190, 2008.
22 Venugopal J and Blanco G: Ouabain enhances ADPKD cell apoptosis *via* the intrinsic pathway. Front Physiol *7*: 107.eCollection, 2016.
23 Hillemanns P, Wang X, Staehle S, Michels W and Dannecker C: Evaluation of different treatment modalities for vulvar intraepithelial neoplasia (VIN): CO(2) laser vaporization, photodynamic therapy, excision and vulvectomy. Gynecol Oncol *100*: 271-275, 2006.
24 Krimbacher E, Zeimet AG, Marth C and Kostron H: Photodynamic therapy for recurrent gynecologic malignancy: a report on 4 cases. Arch Gynecol Obstet *262*: 193-197, 1999.
25 Lele SB, Piver MS, Mang TS, Dougherty TJ and Tomczak MJ: Photodynamic therapy in gynecologic malignancies. Gynecol Oncol *34*: 350-352, 1989.
26 Rettenmaier MA, Berman ML, Disaia PJ, Burns RG and Berns MW: Photoradiation therapy of gynecologic malignancies. Gynecol Oncol *17*: 200-206, 1984.
27 Yamaguchi S, Tsuda H, Takemori M, Nakata S, Nishimura S, Kawamura N, Hanioka K, Inoue T and Nishimura R: Photodynamic therapy for cervical intraepithelial neoplasia. Oncology *69*: 110-116, 2005.
28 Friedl P and Alexander S: Cancer invasion and the microenvironment: plasticity and reciprocity. Cell *147*: 992-1009, 2011.
29 Lee SH, Jeong D, Han YS and Baek MJ: Pivotal role of vascular endothelial growth factor pathway in tumor angiogenesis. Ann Surg Treat Res *89*: 1-8, 2015.
30 Hanahan D and Weinberg RA: Hallmarks of cancer: the next generation. Cell *144*: 646-674, 2011.
31 Wang D and Dubois RN: Eicosanoids and cancer. Nat Rev Cancer *10*: 181-193, 2010.
32 Legler DF, Bruckner M, Uetz-von Allmen E and Krause P: Prostaglandin $E_2$ at new glance: novel insights in functional diversity offer therapeutic chances. Int J Biochem Cell Biol *42*: 1 98-201, 2010.
33 Ciardiello F, Troiani T, Bianco R, Orditura M, Morgillo F, Martinelli E, Morelli MP, Cascone T and Tortora G: Interaction between the epidermal growth factor receptor (EGFR) and the vascular endothelial growth factor (VEGF) pathways: a rational approach for multi-target anticancer therapy. Ann Oncol *17*: 109-114, 2006.
34 Jaffe AB and Hall A: Rho GTPases: biochemistry and biology. Annu Rev Cell Dev Biol *21*: 247-269, 2005.
35 Connolly JO, Simpson N, Hewlett L and Hall A: Rac regulates endothelial morphogenesis and capillary assembly. Mol Biol Cell *13*: 2474-2485, 2002.
36 Hoang MV, Whelan MC and Senger DR: Rho activity critically and selectively regulates endothelial cell organization during angiogenesis. Proc Natl Acad Sci USA *101*: 1874-1879, 2004.
37 Park HJ, Kong D, Iruela-Arispe L, Begley U, Tang D and Galper JB: 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors interfere with angiogenesis by inhibiting the geranylgeranylation of RhoA. Circ Res *91*: 143-150, 2002.
38 Hakoshima T, Shimizu T and Maesaki R: Structural basis of the Rho GTPase signaling. J Biochem *134*: 327-331, 2003.
39 Bryan BA, Dennstedt E, DC, Walshe TE, Noma K, Loureiro R, Saint-Geniez M, Jean-Paul Campaigniac J-P, Liao JK and D'Amore PA: RhoA/ROCK signaling is essential for multiple aspects of VEGF-mediated angiogenesis. FASEB J *24*: 3186-3195, 2010.

*Received April 27, 2017*
*Revised May 25, 2017*
*Accepted May 29, 2017*