JOURNAL OF BIOMEDICAL OPTICS 4(3), 272–275 (JULY 1999)

# PHOTODYNAMIC TREATMENT OF EPITHELIAL TISSUE DERIVED FROM PATIENTS WITH ENDOMETRIAL CANCER: A CONTRIBUTION TO THE ROLE OF LAMININ AND EPIDERMAL GROWTH FACTOR RECEPTOR IN PHOTODYNAMIC THERAPY

**Piotr Ziółkowski,[†] Krzysztof Symonowicz,[†] Beata J. Osiecka,[‡] Jerzy Rabczyński,[†] and Jerzy Gerber***

[†]Departments of Pathology, [‡]Histology and Embryology, and *Gynecology and Obstetrics, Medical University of Wrocław, 50-368 Wrocław, Poland

(Paper PT-001 received Sep. 7, 1997; revised manuscript received May 12, 1999; accepted for publication May 12, 1999. )

## ABSTRACT

Photodynamic therapy (PDT) was used to treat endometrial G1 cancer tissue derived from patients who had undergone a total hysterectomy and bilateral salpingo-oophorectomy. After surgical treatment the cancerous tissue was kept in a medium containing Dulbecco solution, fetal calf serum, and antibiotics. The tissue was then exposed to hematoporphyrin derivative (0.1 mg/ℓ) and 24 h later exposed to light (total light dose—18 J/sq cm). Necrosis depth was evaluated 24 h later using a light microscope. In order to assess the possible role of the basal membrane component laminin, as well as epidermal growth factor receptor susceptibility to PDT, immunohistochemical studies were carried out. Additionally, nucleolar organizer regions evaluation was performed. Our experiment confirmed that PDT results in the necrosis in the treated endometrial cancer, while not affecting the laminin in the cancerous tissue. In contrast, PDT strongly affects the epidermal growth factor receptor and nucleolar organizer regions in cancer cells. We suggest that laminin may contribute to the prevention of cancer dissemination in the cases where PDT has to be repeated, and that after PDT the cells become less susceptible to a mitogen, like, e.g., epidermal growth factor. © *1999 Society of Photo-Optical Instrumentation Engineers.* [S1083-3668(99)00903-X]

**Keywords** photodynamic therapy; endometrial cancer; laminin; epidermal growth factor receptor; nucleolar organizer regions.

## 1 INTRODUCTION

Photodynamic therapy (PDT) has been widely used to treat various malignancies, e.g., cancers of the gynecological tract.[1] Many gynecologic tumors are ''surface'' malignancies and therefore accessible to PDT, which is limited by penetration of delivered light.[2] They are usually early cancers and often recurrent tumors.[2] Small, superficial endometrial cancers (also recurrent) responded well to PDT; therefore hysteroscopical PDT has previously been regarded as a useful tool in gynecology.[2]

Laminin (LM) is a marker of basal membrane integrity and is usually made visible in order to distinguish between invasive and noninvasive carcinomas, e.g., in cervical or skin squamous cancer.[3] Since PDT and hysteroscopical PDT as well can be repeated several times in order to eradicate an entire tumor, it is necessary to know whether or not, after the first PDT attempt, the tumor residuals will disseminate because of damage to the basal membrane.

Epidermal growth factor (EGF) is a 6 kd protein that binds to a 170 kd cell surface EGF receptor (EGFR).[4] EGF, a potent mitogen found in nearly all bodily fluids, is capable of stimulating a variety of cells *in vitro* and *in vivo*, and belongs to the main angiogenesis stimulating factors.[4] EGFR overexpression has been described in a variety of tumor types including those of the gynecological tract.[4] Therefore, we considered EGFR an important factor to be assessed after the initial PDT. Nucleolar organizer regions (NORs) are commonly used markers of tumor proliferation potency.[5] NORs have previously been examined only on an animal tumor

Address all correspondence to Piotr Ziółkowski. Tel: 4871-3209974; Fax: 4871-3281029.

model (*in vivo*) where they showed a significant decrease after PDT.[6]

To our knowledge, the status of LM and EGFR in PDT has not previously been studied.

## 2 MATERIALS AND METHODS

### 2.1 PHOTOSENSITIZER

Hematoporphyrin derivative (HpD) was purchased from Porphyrin Products, Inc., Logan, UT. The sensitizer was dissolved in physiological saline and made alkaline with 0.05 M NaOH to a final dose of 0.1 mg/ℓ. The solution of HpD was prepared just before use and added to the medium containing cancer tissue, i.e., on the day of hysterectomy.

### 2.2 LIGHT SOURCE

Penta Lamps Teclas (CH) were used. The light (wavelength: 620–640 nm) in a total light dose of 18 J/sq. cm (fluence rate—75 mW) was passed after 24 h incubation of the cancer tissue in a mixture of medium and solution of HpD, i.e., 24 h after surgery.

### 2.3 CANCER TISSUE

Cancer tissue was derived from females (mean age—54) with endometrial cancer (number of examined cases—10; histological grade—1). The patients underwent standard surgical hysterectomy with bilateral adnexectomia. The cancerous tissues obtained were divided into two parts. One part (I) from the ten cases mentioned was fixed in formalin, paraffin embedded, and stained with hematoxylin–eosin in order to assess a spontaneous necrosis. The other part (II) from the ten cancer cases was placed in 10 ml of medium which kept the cells alive. The medium consisted of fetal calf serum: 1 ml, Dulbecco solution: 9 ml (both obtained from Institute of Immunology and Experimental Therapy, Wrocław, Poland), and antibiotics (penicillin: 100 U/ml, streptamycin: 100 mg/ml, and gentamycin: 200 mg/ml, obtained from Jelfa, Poland). The PDT treated samples (II) from the ten cases were fixed in formalin 24 h after exposure to the light, paraffin embedded, cut into slices, and stained with H-E (in order to assess the PDT induced necrosis), immunohistochemically (for LM, EGFR) or with $AgNO_3$ for NORs. The control group for comparison with the cancer tissues (II) comprised endometrial cancer tissues derived from three patients treated surgically. These cancer tissues were also placed in medium, but they were not subjected to PDT. They were then processed immunohistochemically.

### 2.4 IMMUNOHISTOCHEMICAL ASSAY

Laminin (Sigma Immunochemicals, St. Louis, MO, USA), diluted 1:100 and epidermal growth factor receptor (Novócastra Laboratories, Ltd, Newcastle, UK) diluted 1:200 were used as monoclonal antibodies. The avidin–biotinylated alkaline phosphatase complex (ABC kit), (DAKO, Denmark) was then used together with diaminobenzidine to make visible the antigens.

The NORs were stained by the routine procedure using $AgNO_3$. All samples were examined under the light microscope.

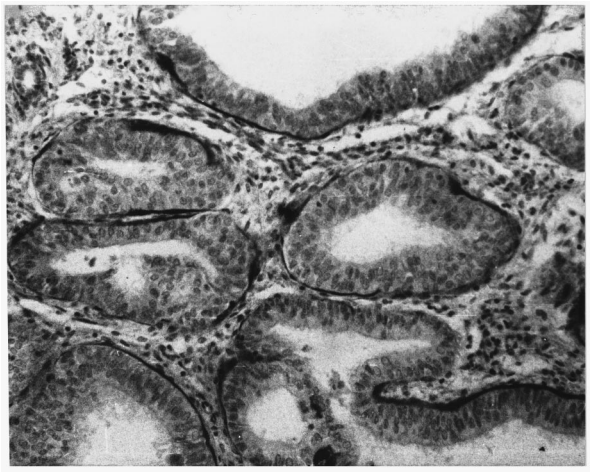

**Fig. 1** Endometrial cancer treated with PDT. Laminin is present as a dark, continuous lining of cancer epithelium with single gaps. No invasion of cancer cells beyond the basal membrane can be seen.

## 3 RESULTS

The samples obtained from the ten patients with endometrial cancer showed no spontaneous necrosis upon staining with H-E.

The ten samples treated with PDT and stained immunohistochemically showed no damage to the basal membrane (BM) component—laminin, in comparison with the three control samples. This BM component was mostly seen, in both the experimental and control groups, as a continuous structure which surrounded the nests of cancer cells. In some parts of the PDT treated and control tumors the laminin seemed to be broken (gaps), but no dissemination of cells beyond the basal membrane was observed (Figure 1). It must be stressed that all the samples examined revealed very similar patterns of laminin expression.

The samples treated with PDT showed a very significant decrease in the expression of the EGFR. Indeed most areas of the ten cancer tissues showed no expression of EGFR at all (Figure 2). In contrast, in the three control samples, the EGFR expression was still very high (Figure 3).

Next, the staining for NORs showed a very low expression of this proliferation marker after treatment with PDT (25% of cancer cells), whereas in the control samples the NOR expression was very strong with almost 85% of the cancer cells showing numerous NORs.

Photodynamic therapy also resulted in an enhancement of cell necrosis in almost the entire cancer tissue of the ten samples of endometrial cancer

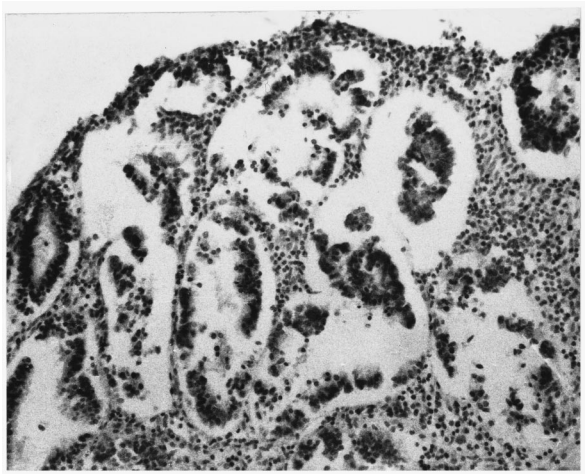

**Fig. 2** Endometrial cancer treated with PDT. A very significant decrease of epidermal growth factor receptor expression is seen.

studied. The untreated samples did not reveal any necrosis of the cells after 2 days of incubation in the medium. Briefly, PDT caused a significant enhancement of necrosis which was correlated with very low expressions of both EGFR and NORs, and slight, if any, damage to the laminin.

## 4 DISCUSSION

Endometrial adenocarcinoma recurrent in the vulva and groin has previously been treated by PDT.[2] The superficial, small, labial recurrence of that cancer responded very well to PDT, whereas the study showed that poorer results were obtained after PDT treatment of squamous cell carcinoma recurrences.[2] In another study, Koren and Alth[1] treated seven patients with endometrial carcinomas at the FIGO 1a stage, i.e., restricted to the endometrium, by use of PDT, gaining a high ratio of complete responses.

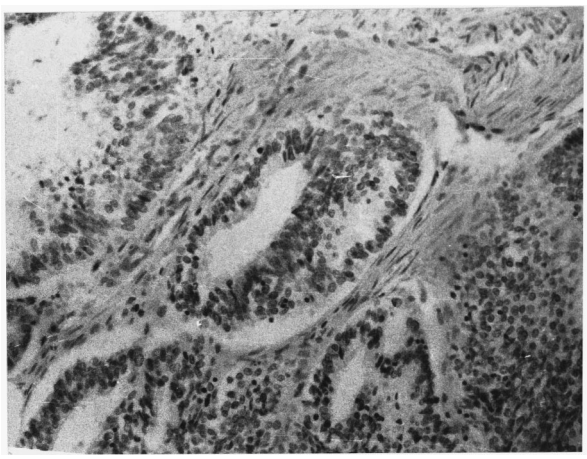

**Fig. 3** Endometrial cancer from the control group. The expression of epidermal growth factor receptor is still very high.

Corti et al. used PDT in the treatment of locoregional recurrences of cervical and five cases of endometrial adenocarcinoma.[7]

The drawbacks of conventional therapies of gynecological malignancies are caused by severe side effects such as the destruction of physiological tissues and structures by surgery or radiation.[8] Our study confirmed that very important markers of tumor invasion, i.e., laminin (and BM as well), are not affected by PDT. We did not see any significant difference between the expression and maturity of laminin in the two groups studied (single gaps and loss of integrity were seen with the same frequency in both). Thus, laminin might contribute to the prevention of cancer dissemination in those cases where PDT has to be repeated two or more times, especially in G1 cancers. It has been suggested that overexpression of c-erb-B2 (HER-2/neu) oncogene is related to the more advanced stages of endometrial cancer, the absence of estrogen receptors, and a worse prognosis.[9,10] Mutations that can affect the receptor function are gene overexpression or amplification and these render the cell oversensitive to the growth factor.[11] Thus, the mutant receptors deliver a continuous mitogenic signal to the cell.[11] In our study we have assessed the EGFR expression, which was found to be decreased following PDT. This is a very important finding; briefly it means that after PDT the cells become less susceptible to a mitogen, i.e., to EGF. Even those cells which survived after a single PDT could not be stimulated by EGF since they did not express the proper receptors. A decrease in the number of NORs in cancer cells was expected, and this is in agreement with our previous *in vivo* results on an animal tumor model.[6] It also correlates with the observed intensity of endometrial cancer necrosis.

Hysteroscopical PDT may be a useful alternative to total resection in patients with well-differentiated superficial, endometrial adenocarcinomas and/or who are considered poor surgical risks.[7] It can be repeated several times without danger of dissemination and with a decrease in cell proliferation potency. In general, a better response can be obtained for small, superficial tumors.[7] Previous studies[2] and our results confirm that other possibilities may include presurgical shrinkage of large tumors.

## 5 CONCLUSIONS

1. The laminin may contribute to the prevention of cancer dissemination in those cases where PDT has to be repeated.
2. After PDT the endometrial adenocarcinoma cells become less susceptible to a mitogen like, e.g., epidermal growth factor.
3. The decrease in the number of NORs after PDT indicates lower proliferative potentials of cancer cells.

## REFERENCES


1. H. Koren and G. Alth, ''Photodynamic therapy in gynecologic cancer,'' *J. Photochem. Photobiol., B* **36**, 189–191 (1996).
2. M. A. Rettenmaier, M. L. Berman, P. J. DiSaia, R. G. Burns, and M. W. Berns, ''Photoradiation therapy of gynecologic malignancies,'' *Gynecol. Oncol.* **17**, 200–206 (1984).
3. S. J. Forster, I. C. Talbot, and D. R. Critchley, ''Laminin and fibronectin in rectal adenocarcinoma: relationship to tumor grade, stage and metastasis,'' *Br. J. Cancer* **50**, 51–61 (1984).
4. W. J. Gullick, ''Prevalence of aberrant expression of the epidermal growth factor receptors in human cancers,'' *Br. Med. Bull.* **47**, 87–90 (1991).
5. M. Jeleń (unpublished report).
6. P. Ziółkowski, J. Milach, K. Symonowicz, B. Zawirska, and T. Szkudlarek, ''Photodynamic therapy affects heat-shock-protein-72 intracellular translocation in *in vivo* conditions,'' *Acta Bio-Optica Inform. Med.* **1**, 89–93 (1995).
7. L. Corti, R. Mazzarotto, S. Belfontali, C. De Luca, C. Baiocchi, C. Boso, and F. Calzavara, ''Photodynamic therapy in gynecological neoplastic diseases,'' *J. Photochem. Photobiol., B* **36**, 193–197 (1996).
8. H. Soma and S. Nutahara, ''Cancer of the female genitalia,'' in *Lasers and Hematoporphyrin Derivative in Cancer*, Y. Hayata, and T. J. Dougherty, Eds., pp. 97–109, Igaku-Shoin, Tokyo (1983).
9. A. Berchuck, G. Rodriquez, and R. Kinney, ''Overexpression of HER-2/neu in endometrial cancer is associated with advanced stage disease,'' *Am. J. Obstet. Gynecol.* **164**, 15–21 (1991).
10. H. Sasano, J. Comerford, and D. S. Wilkinson, ''Serous papillary adenocarcinoma of the endometrium—analysis of pro-oncogene amplification, flow cytometry, estrogen and progesterone receptors and immunohistochemistry,'' *Cancer (N.Y.)* **65**, 1545–1551 (1990).
11. J. Markowska, ''Oncogenes and tumor suppressor genes associated with gynecologic malignancies,'' *Centr.-Eur. J. Immunol.* **21**, 211–221 (1996).