[CANCER RESEARCH 62, 6381–6384, November 15, 2002]

Advances in Brief

Growth Inhibition of Cervix Carcinoma Cells *in Vivo* by Endothelin A Receptor Blockade[1]

Anna Bagnato, Alessia Cirilli, Debora Salani, Paola Simeone, Antonio Muller, Maria Rita Nicotra, Pier Giorgio Natali, and Aldo Venuti[2]

Laboratories of Virology [A. C., P. S., A. M., A. V.], Molecular Pathology and Ultrastructure [A. B., D. S.], Immunology [P. G. N.], Regina Elena Cancer Institute, and Biotechnology Department Consiglio Nazionale delle Richerche [M. R. N.], 00158 Rome, Italy

Abstract

In human papillomavirus (HPV)-positive cervical cancer cells, the endothelin A receptor (ET_AR) mediates an endothelin-1-induced mitogenic effect, thus representing a relevant target for antitumor therapy. Here, we describe the complete inhibition of human cervix carcinoma growth by blocking the ET_AR. In nude mice, the ET_AR-selective antagonist atrasentan inhibits the growth and the neoangiogenesis of cervical carcinoma cell xenografts. Two cycles of treatment completely revert tumor growth. Atrasentan displays additive effects when administered in combination with the cytotoxic drug paclitaxel. These results demonstrate that by inhibiting cell proliferation and angiogenesis, this small molecule may help to control cervical cancer by either monotherapy or combination therapy.

Introduction

Cervical cancer is the second most common cancer in women worldwide (breast cancer is the most common) and the leading cause of cancer-related death among women in developing countries. Biological compounds with low toxicity may help to improve current cervical cancer therapies. The family of ETs[3], including ET-1, ET-2, and ET-3, are 21-amino acid peptides exerting many biological effects (1). Two major receptor subtypes belonging to the G protein-coupled family receptors mediate ET signals: the ET_AR, which binds ET-1 and ET-2 with high affinity and ET-3 with low affinity; and the ET_BR, which binds all ET isopeptides with equal affinity (2). ET-1 induces cell proliferation directly or synergistically with other growth factors that are relevant in cancer progression. It has been proposed that ET-1 exerts autocrine or paracrine action on neoplastic and surrounding stromal cells, contributing to development and progression of a variety of malignancies (2, 3). Engagement of ET_AR by ET-1 triggers activation of tumor proliferation (3–7), VEGF-induced angiogenesis, (8, 9) invasiveness, (10), and inhibition of paclitaxel-induced apoptosis (11, 12). Human keratinocytes express ET_AR and produce ET-1, which sustains an autocrine growth response (13). HPV can immortalize and transform human keratinocytes that secrete ET-1 and exhibit a 2-fold increase in ET_AR compared with parental cells in the absence of recycling alteration (14). ET-1 induces an increased growth response in these cell lines (which can proliferate in the absence of any growth factor; Ref. 15). ET-1 and its ET_AR are also overexpressed in HPV-associated cervix carcinoma cells (7). Binding studies showed that these transformed cells express an increased number of functional ET_AR and that ET-1 (but not ET-3) stimulates a marked dose-dependent increase in [^{3}H]thymidine incorporation compared with the normal counterpart. This proliferative effect is selectively blocked by an ET_AR antagonist, whereas an ET_BR antagonist has no effect. These results demonstrate that ET-1 is involved in the growth of HPV-associated carcinoma cells overexpressing ET_AR and that these receptors are clinically relevant targets for antitumor therapy. In this study, we provide evidence that a selective antagonist of the ET_AR, ABT-627 (16) is able to inhibit the *in vivo* growth of HPV-associated cervical carcinoma cells and to potentiate cytotoxic treatment in combination with paclitaxel. This inhibition is associated with a reduced density of tumor-associated microvessels. These results demonstrate the antiangiogenic and antitumor effect of this selective ET_AR antagonist in cervical carcinoma and support the clinical use of ABT-627 in monotherapy or in combination with cytotoxic drugs.

Materials and Methods

Cell Lines. Cervical carcinoma-derived cell lines, CaSki and C33A, were purchased from American Type Culture Collection. CaSki cells were maintained in RPMI/10% FCS (Invitrogen, Milan, Italy) and C33A cell lines in MEM/10% FCS (Invitrogen). CaSki cells are HPV16-positive, produce ET-1, and express mRNA for ET_ARs and ET_BRs. C33A cells, which are HPV negative, do not produce ET-1 and express only mRNA for ET_BRs (7).

Cell Proliferation Assay. Cells were seeded in 96-well plates at a density (2×10^4 cells/well) allowing an exponential growth rate. To induce the inhibition of cell proliferation, paclitaxel (Sigma-Aldrich, Milan, Italy) at 100 nM was added to the cultures for 4 h. ET-1 was added at a concentration of 100 nM. The ET_AR antagonist was used at 1 μM by adding the compound 15 min before paclitaxel and/or ET-1. Twenty-four h later, the cellular monolayers were stained with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma-Aldrich) for 2 h. The reduced dye was then eluted by isopropanol, and cell metabolic activity was evaluated by measuring the absorbance at $A^{540-620}$ in a laser microdensitometer. Eight replicas for each point were assayed.

Tumor Cell Xenografts. Female nu^+/nu^+ mice (Charles River, Cologno Monzese, Italy) were 8 weeks old. Single cell suspension of 1.5×10^6 of CaSki and C33A cells with a viability $> 95\%$ was injected s.c. into the flank of nude mice. Palpable tumors were detected $\sim$7 days after cell injection. Tumor burden was measured with a caliper and calculated as length $\times$ width$^2 \times 0.5$.

Treatment Schedule. We injected 200 μl of a solution containing 1 or 5 mg/ml of ABT-627 (Abbott Laboratories, Chicago, IL) dissolved in $NaHCO_3$ 0.25 N, i.p. once a day for 21 days, corresponding to a dosage of 2 or 10 mg/kg/day, respectively. The treatment was started at various times after the xenograft. Control mice were given injections in the same way with 200 μl of vehicle. For the combined therapy, the ABT-627 treatment was administered in combination with paclitaxel. A dosage of 10 mg/kg of paclitaxel (Bristol Myers, Pomezia, Italy) dissolved in cremophor EL® (Sigma-Aldrich) was injected three times at 3-day intervals in the tail vein of the mice starting 7 days after the xenograft. Control mice received injections of the cremophor EL alone.

Received 8/9/02; accepted 10/2/02.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was partially supported by Associazione Italiana Ricerca sul Cancro and Ministero della Salute.

[2] To whom requests for reprints should be addressed, at Laboratory of Virology, Regina Elena Cancer Institute, via delle Messi d'Oro 156, 00158 Rome, Italy. Phone: 39-06-5266-2521; Fax: 39-02-5266-2505; E-mail: venuti@ifo.it.

[3] The abbreviations used are: ET, endothelin; ET_AR, endothelin A receptor; ET_BR, endothelin B receptor; VEGF, vascular endothelial growth factor; HPV, human papillomavirus; ABT-627, atrasentan.

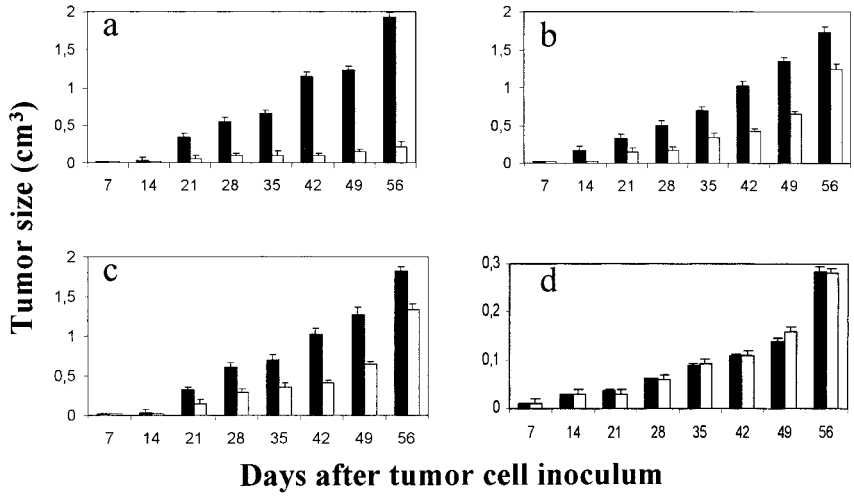

Fig. 1. ABT-627 treatment of cervical carcinoma xenografts. Female nu^+/nu^+ mice were given injections of 1.5×10^6 CaSki (*a–c*) or C33A cells (*d*). ABT-627 treatment at 2 mg/kg/day for 21 days was started the same day of tumor cell transplant (*a* and *d*) or 3 (*b*) or 7 days thereafter (□). In the last condition, a palpable tumor was present in all injected mice. The control animals (■) were given injections of the vehicle, $NaHCO_3$ 0.25 N. Tumor volume was recorded by measuring dimension with a caliper and calculating as length × width² × 0.5. *Columns*, means; *bars*, SD.

Vessel Count. Four weeks after the end of treatment, mice were sacrificed, and tumors were collected. Consecutive 4-μm sections of frozen tissue were stained by H&E or immunostained for CD31 by a rat monoclonal antimouse CD31 [specific for mouse endothelial cells; a gift from Dr. Alberto Mantovani (Istituto Ricerche Farmacologiche Mario Negri, Milan, Italy)]. Immunohistochemical staining was performed by the immunoperoxidase technique (Vector Laboratories, Burlingame, CA). The microvessel number was counted by two different observers in tissue areas, which included higher microvessels density (hot spot).

Statistical Analysis. All experiments were analyzed by the Student's test performed by the Instat software (GraphPad Software, Inc., San Diego, CA). All of the *P*s resulted from the use of two-sided tests and were considered significant when <0.05.

Results

Growth Inhibition of Cervical Carcinoma Xenograft. Although both cell lines CaSki and C33A were able to produce tumors in nude mice, the CaSki cell tumors had increased tumor mass compared with C33A cells. This difference in tumor burden might reflect the major ability of CaSki cells to proliferate in conditions of low growth factor stimulation (7).

ABT-627 was able to affect the tumor implant at the 2 mg/kg/day dosage, depending on the administration start time. This dosage was chosen because it corresponded to that used in human clinical trials (16). Early treatment (at 0 and 3 days from cell injection) as well as late treatment (at 7 days, when the tumors were already palpable) with the same dosage (2 mg/kg/day) were effective in reducing the size of tumors produced by CaSki cells and in delaying tumor growth (Fig. 1, *a–c*).

The same dosage of the antagonist ABT-627 that was effective on CaSki xenografts in reducing tumor mass, with maximum activity at early treatment, had no effect on C33A xenografts, as expected because of the absence of ET_AR in this cell line (Fig. 1*d*).

As the single treatment with ABT-627 produced a reduction in tumor mass that was more evident during the treatment and shortly thereafter, a two-cycle treatment was given to the mice. Two cycles of 21-day treatment (with 7-day-intervals) was started when palpable tumors were present in the flank of the mice. This double treatment had a dramatic effect on tumor growth and caused recovery from tumor in 80% of treated mice (Fig. 2). In the remaining animals, the tumor growth inhibition was higher than that produced by a single cycle of treatment. Higher dosages of ABT-627 (10 mg/kg/day) did not increase the reduction of the tumor mass but affected the survival time of the treated animals with a higher survival probability (data not shown).

Blood Vessel Density. ET-1 triggers activation of VEGF-induced angiogenesis by stimulating VEGF production through ET_AR-mediated signaling (9). The VEGF-induced angiogenesis may be evaluated by the number of blood vessels within the tumor. ABT-627, at the same dosage affecting tumor growth, produced a statistically significant reduction in the number of blood vessels within the tumor (Fig. 3). In the hot-spot sites of control tumors, 19 ± 3.49 vessels were detected, whereas in ABT-627-treated tumors, only 8 ± 3.44 vessels were present ($P = 0.006$).

***In Vitro* Cooperation between ABT-627 and Paclitaxel in Inhibiting Carcinoma Cell Proliferation.** We have previously demonstrated that ABT-627 is more effective than BQ 123 in inhibiting proliferation of CaSki cells (17). A dramatic growth inhibition occurred when these cells were treated *in vitro* with the chemotherapeutic agent paclitaxel, and this effect was counteracted by the presence of ET-1. The metabolic activity of CaSki cells, which was reduced to ~50% by paclitaxel treatment, was increased toward the level of the untreated cells by the presence of ET-1 (Fig. 4*A*). Treatment with the ET_AR antagonist, ABT-627, counteracted the effects of ET-1 and restored tumor sensitivity to paclitaxel. Simultaneous treatment with

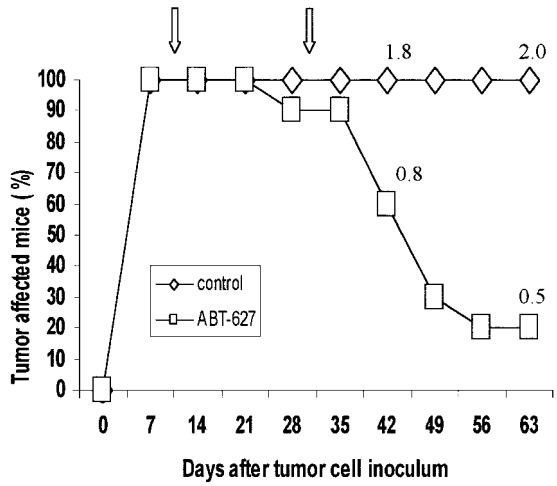

Fig. 2. Recovery from tumor development by two cycles of ABT-627 treatments. CaSki cells (1.5×10^6) were inoculated into nude mice. Seven days after a palpable tumor was present, treatment with ABT-627 at 2 mg/kg/day for 21days was started. The control animals were treated with the vehicle $NaHCO_3$ 0.25 N. Seven days after the end of the first treatment, a new treatment cycle with the same dosage and time was undertaken. The tumor development was monitored by palpation and measured with a caliper. The *arrows* indicate the starting point of treatments. The *numbers over the symbols* are the means of the tumor volume of the affected animals.

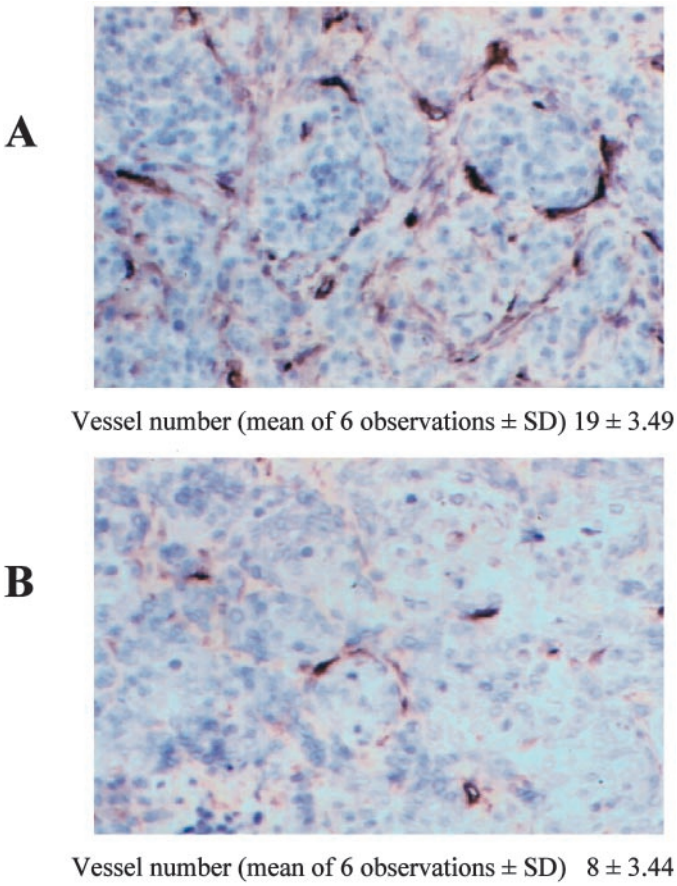

Fig. 3. Blood vessel density. Four weeks after the end of treatment, mice were sacrificed, and tumors were collected. Sections (4 μm) were immunostained for a specific marker of mouse endothelial cells by a rat monoclonal antimouse CD31. The microvessel number was counted by two different observers in tissue areas, which included higher microvessel density than the rest of the tissue (hot-spot). The mean value ± SD of the microvessel number is indicated below each panel. *A*, untreated tumor; *B*, tumor treated with ABT-627.

ABT-627 and paclitaxel significantly increased the inhibition of cell proliferation by blocking the action of endogenous ET-1 produced by CaSki cells (Fig. 4*A*).

***In Vivo* ABT-627 and Paclitaxel Cooperation.** CaSki xenografts were highly sensitive to paclitaxel treatment, with a dramatic reduction of tumor mass. Nevertheless, combined treatment with the antagonist ABT-627 was able to induce a better response to the paclitaxel treatment by further reducing the tumor mass by an additional 70% (Fig. 4*B*).

Discussion

Changes in the homeostasis of growth factor-induced physiological signaling may lead to unbalanced cell growth. Signaling through the ET_AR activates molecular pathways leading to tumor cell proliferation, neovascularization, invasiveness, and protection against apoptosis (4–12). The ET-1 signaling pathway is up-regulated in various human tumors, including ovarian, breast, and prostate cancer (6, 18, 19). The overproduction of ET-1 and the up-regulation of the autocrine loop mediates by ET_AR in HPV-associated cervical carcinoma cells indicate that this receptor could be used as a target for therapy.

Consequent to this hypothesis, compounds that antagonize the action of ET-1 by blocking ET_AR would be able to affect the growth of cervical carcinoma xenografts in nude mice. In this study, we have investigated the action of the ET_AR antagonist ABT-627 on the growth of cervical cancer xenografts in monotherapy as well as in association with the chemotherapeutic compound, paclitaxel.

ABT-627 is a nonpeptide-selective antagonist of ET_AR that is in use in clinical trials on metastatic adenocarcinoma of the prostate. This compound was more effective than the peptide antagonist, BQ123, in blocking proliferation and growth of HPV-associated cervical cancer cells *in vitro* (17). As for other growth factor receptor inhibitors, like those for EGFR, we were expecting to find inhibitory effects against the cervical carcinoma xenograft only during treatment, with a substantial tumor regrowth upon the termination of treatment (20). Surprisingly, ABT-627 treatment at a dosage of 2 mg/kg/day for 21 days caused a marked inhibition and subsequent delay of tumor growth for several weeks after treatment in the absence of any toxic effect or weight loss. Early treatment influenced the tumor implant and caused almost complete inhibition of carcinoma cell xenograft growth. The effect of this selective ET_AR inhibitor confirms that in HPV-infected cervical carcinoma cells, the ET_AR mediates autocrine tumor growth stimulation. The lack of effect of ABT-627 in C33A xenografts is indicative of its specificity, as it is known that C33A cells do not express the ET_AR and are HPV negative. Higher dosages of ABT-627 (10 mg/kg/day) did not increase the reduction of

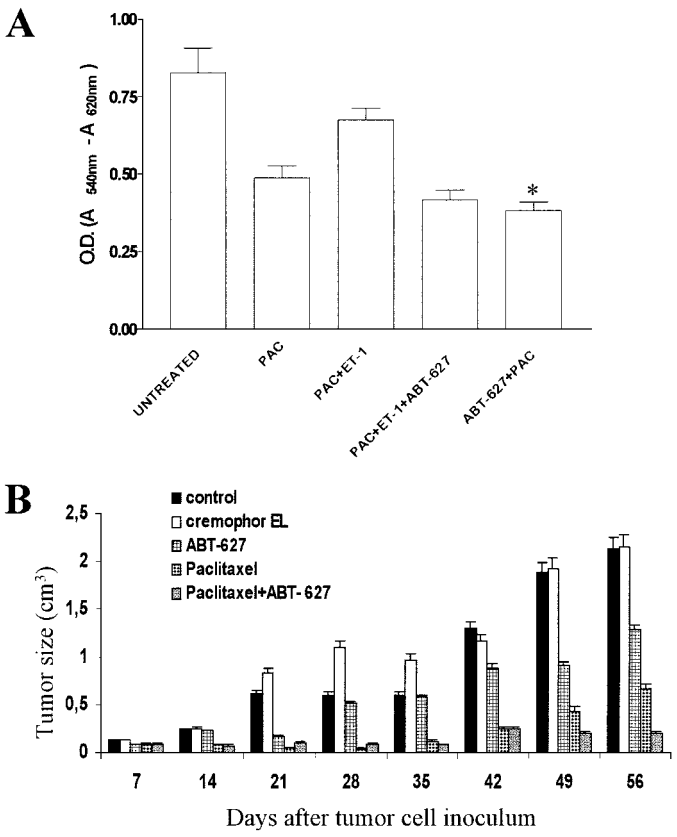

Fig. 4. Combination therapy with ABT-627 and Paclitaxel. *A*, *in vitro*. The metabolic activity of CaSki cell was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide test 24 h after the treatment. To induce growth inhibition in serum-starved CaSki cells, paclitaxel at 100 nM was added to the cultures for 4 h. In some conditions, ET-1 at 100 nM was added. In the combined treatment, ABT-627 at 1 μM was added 15 min before paclitaxel and/or ET-1. *Columns*, mean of three independent experiments, each with sextuplicate wells; *bars*, SD. *, $P < 0.02$ compared with paclitaxel treatment. *B*, *In vivo*. Nude mice were given injections of 1.5×10^6 CaSki cells, and tumor development was monitored by measuring tumor volume with a caliper. ABT-627 at 2 mg/kg/day was administrated i.p. for 21 days, starting 7 days after tumor transplant when a palpable mass was present. Control mice received injections of $NaHCO_3$ 0.25 N. For combination therapy, a dosage of 10 mg/kg of paclitaxel dissolved in cremophor EL was injected three times at 3-day intervals in the tail vein of the mice starting the same day of ABT-627 treatment. To exclude any biological activity of the vehicle, a group of mice was treated with cremophor EL without paclitaxel.

tumor mass, indicating that the lower doses can saturate the receptors on the tumor cell membrane. The action of high doses on the survival time of the treated animals may be explained by other systemic actions of the ET_AR antagonists on the organism.

ET-1 causes activation of VEGF-induced angiogenesis by stimulating VEGF production (8, 9). This action is mediated by the ET_AR, and therefore specific antagonists of this receptor may affect the tumor vascularization. Indeed, ABT-627 produced a statistically significant reduction in the number of blood vessels within the tumor, consistent with the similar role of ET-1 in neovascularization of ovarian carcinoma (9).

ABT-627 seems effective in controlling at least two main aspects of tumor growth: proliferation and neovascularization. However, several weeks after the end of the treatment, small tumors were still present. By introducing a second cycle of therapy, the effect on cervical carcinoma xenograft was more pronounced: 80% of treated animals recovered from the tumor and, in the remaining animals, the tumor volume was reduced at low levels with respect to the control. The cured animals remained tumor-free until the end of the observation (12 months). This is the first observation that two cycles of targeted therapy are able to completely revert tumor growth, at least in nude mouse models.

As stated before, ET-1 protects against paclitaxel-induced apoptosis in ovarian tumor cells through its type A receptor (12). In CaSki cells, paclitaxel treatment caused an inhibition of cell growth *in vitro* that was counteracted by the presence of exogenous ET-1. The presence of ABT-627 affects the action of ET-1 and sensitizes the tumor cells to the activity of paclitaxel. In nude mice, the combined treatment was therefore investigated. CaSki xenografts were highly sensitive to paclitaxel treatment. Nevertheless, ABT-627 treatment was able to increase the tumor growth inhibition by a single cycle of therapy.

Previous studies by competitive binding data revealed that HPV-positive cervical carcinoma cells predominantly express functional ET_AR, supporting the hypothesis that the expression and function of ET_AR in malignant cervical cells is likely to become the predominant form, representing a relevant mechanism in tumor proliferation (7). The present data reinforce this hypothesis and indicate that prolonged treatment with ET_AR antagonists should be an effective therapy. The ET_AR antagonist not only inhibits carcinoma cell proliferation but also interferes with neovascularization, presumably by decreasing VEGF production (9). These findings indicate that a single compound may act against several targets of the neoplastic disease, including the potentiation of cytotoxic drug action.

Taken together, our results demonstrate that *in vivo*, at least in the mouse model, the ET-1/ET_AR autocrine loop is a target of gene-based therapy and that such therapy is effective alone or in combination with cytotoxic drugs in inhibiting the growth of HPV-associated neoplasia.

Acknowledgments

We thank the entire staff of the animal house for animal studies.

References

1. Yanagisawa, M., Kurihara, H., Kimura, S., Tomobe, Y., Kobayashi, M., Mitsui, Y., Yazaki, Y., Goto, K., and Masaki, T. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature (Lond.), *332:* 411–415, 1989.
2. Sakurai, T., Yanagisawa, M., Takuwa, Y., Miyazaki, H., Kimura, S., Goto, K., and Masaki, T. Cloning of a cDNA encoding a non-isopeptide-selective subtype of the endothelin receptor. Nature (Lond.), *348:* 732–735, 1990.
3. Bagnato, A., and Catt, K. J. Endothelin as autocrine regulators of tumor cell growth. Trends Endocrinol. Metab., *9:* 378–383, 1998.
4. Pagotto, U., Arzberger, T., Hopfner, U., Sauer, J., Renner, U., Newton, C. J., Lange, M., Uhl, E., Weindl, A., and Stalla, G. K. Expression and localization of endothelin-1 and endothelin receptors in human meningiomas. Evidence for a role in tumoral growth. J. Clin. Investig., *96:* 2017–2025, 1995.
5. Wu-Wong, J. R., Chiou, W., Magnuson, S. R., Bianchi, B. R., and Lin, C. W. Human astrocytoma U138MG cells express predominantly type-A endothelin receptor. Biochim. Biophys. Acta, *1311:* 155–163, 1996.
6. Bagnato, A., Salani, D., Di Castro, V., Wu-Wong, J. R., Tecce, R., Nicotra, M. R., Venuti A., and Natali, P. G. Expression of endothelin-1 and endothelin A receptor in ovarian carcinoma: evidence for an autocrine role in tumor growth. Cancer Res., *59:* 1–8, 1999.
7. Venuti, A., Salani, D., Manni, V., Poggiali, F., and Bagnato, A. Expression of endothelin-1 and endothelin A receptor in HPV-associated cervical carcinoma: new potential targets for anticancer therapy. FASEB J., *14:* 2277–2283, 2000.
8. Salani, D., Taraboletti, G., Rosanò, L., Di Castro, V., Borsotti, P., Giavazzi, R., and Bagnato, A. Endothelin-1 induces an angiogenic phenotype in cultured endothelial cells and stimulates neovascularization *in vivo*. Am. J. Pathol. *157:* 1703–1711, 2000.
9. Salani, D., Di Castro, V., Nicotra, M. R., Rosanò, L., Tecce, R., Venuti, A., Natali, P. G., and Bagnato, A. Role of endothelin-1 in neovascularization of ovarian carcinoma. Am. J. Pathol., *157:* 1537–1547, 2000.
10. Rosanò, L., Varmi, M., Salani, D., Di Castro, V., Spinella, F., Natali, P. G., and Bagnato, A. Endothelin-1 induces tumor proteinase activation and invasiveness of ovarian carcinoma cells. Cancer Res., *61:* 8340–8346, 2001.
11. Eberl, L. P., Valdenaire, O., Saintgiorgio, V., Jeannin, J. F., and Juillerat-Jeanneret, L. Endothelin receptor blockade potentiates FasL-induced apoptosis in rat colon carcinoma cells. Int. J. Cancer, *86:* 182–187, 2000.
12. Del Bufalo, D., Di Castro, V., Biroccio, A., Varmi, M., Salani, D., Rosanò, L., Trisciuoglio, D., Spinella, F., and Bagnato, A. Endothelin-1 protects ovarian carcinoma cells against paclitaxel-induced apoptosis: requirement for Akt activation. Mol. Pharmacol., *61:* 524–532, 2002.
13. Bagnato, A., Venuti, A., Di Castro, V., and Marcante, M. L. Identification of the ETA receptor subtype that mediates endothelin induced autocrine proliferation of normal human keratinocytes. Biochem. Biophys. Res. Commun., *209:* 80–86, 1995.
14. Venuti, A., Marcante, M. L., Flamini, S., Di Castro, V., and Bagnato, A. The autonomous growth of Human Papillomavirus type 16 immortalized keratinocytes is related to endothelin-1 autocrine loop. J. Virol., *71:* 6898–6904, 1997.
15. Venuti, A., Salani, D., Poggiali, F., Manni, V., and Bagnato, A. The E5 oncoprotein of human papillomavirus type 16 enhances the endothelin-1 induced keratinocytes growth. Virology, *248:* 1–5, 1998.
16. Verhaar, M. C., Grahn, A. Y., Van Weerdt, A. W., Honing, M. L., Morrison, P. J., Yang, Y. P., Padley, R. J., and Rabelink, T. J. Pharmacokinetics and pharmacodynamic effects of ABT-627, an oral ETA selective endothelin antagonist, in humans. Br. J. Clin. Pharmacol., *49:* 562–573, 2000.
17. Venuti, A., Salani, D., Cirilli, A., Simeone, P., Muller, A., Flamini, S., Padley, R., and Bagnato, A. Endothelin receptor blockade inhibits the growth of human papillomavirus-associated cervical carcinoma. Clin. Sci. (Lond.), *103:* 310S–313S, 2002.
18. Ciardiello, F., Caputo, R., Bianco, R., Damiano, V., Somatico, G., De Placido, S., Bianco, A. R., and Tortora, G. Antitumor effect and potentiation of cytotoxic drugs activity in human cancer cells by ZD-1839 (Iressa), an epidermal growth factor receptor-selective tyrosine kinase inhibitor. Clin. Cancer Res., *6:* 2053–2063, 2000.
19. Yamashita, J., Ogawa, M., Egami, H., Matsuo, S., Kiyohara, H., Inada, K., Yamashita, S., and Fujita, S. Abundant expression of immunoreactive endothelin-1 in mammary phyllodes tumor: possible paracrine role of endothelin-1 in the growth of stromal cells in phyllodes tumor. Cancer Res., *52:* 4046–4049, 1992.
20. Nelson, J. B., Hedican, S. P., George, D. J., Reddi, A. H., Piantadosi, S., Eisenberger, M. A., and Simons, J. W. Identification of endothelin-1 in the pathophysiology of metastatic adenocarcinoma of the prostate. Nat. Med., *1:* 944–949, 1996.

Growth Inhibition of Cervix Carcinoma Cells *in Vivo* by Endothelin A Receptor Blockade

Anna Bagnato, Alessia Cirilli, Debora Salani, et al.

Cancer Res 2002;62:6381-6384.