Drugs of the Future 2003, 28(10): 983-989

CCC: 0377-8282/2003

Review Article

Therapies for cancer targeting endothelin receptors

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CONTENTS

Abstract

The endothelin (ET) axis, which includes ET-1, ET-2, ET-3 and the ET receptors, ET_A and ET_B, plays an important physiological role in normal tissue, acting as a modulator of vasomotor tone, tissue differentiation and development, cell proliferation and hormone production. Recent investigations into the role of the ET axis in mitogenesis, apoptosis inhibition, bone remodeling, invasiveness and angiogenesis have provided evidence of the importance of ET-1 in cancer. Data suggest that ET-1 plays a significant role in the growth and progression of a variety of tumors. Development of ET-1 receptor antagonists has provided a better understanding of the ET axis in cancer pathogenesis and suggests an important role for these antagonists in novel therapeutic interventions. The increasing evidence that the ET_A receptor plays a role in the progression of human cancer has led to an extensive search for antagonists that selectively block the ET_A receptor. Atrasentan is one such antagonist that is orally bioavailable and has suitable pharmacokinetic and toxicity profiles for clinical use. Preliminary data from clinical trials of atrasentan in patients with prostate cancer are encouraging. The role of the ET axis and the therapeutic relevance of ET-1 receptor antagonists in a range of malignancies requires further investigation.

Introduction

The endothelin (ET) axis exerts potent effects on normal cells through a variety of cell processes such as tissue differentiation, repair and growth. The ETs comprise a family of 3 small (21-amino acid) peptides: ET-1, ET-2 and ET-3 (3). Endothelins exert their effects in the body by binding to cell surface ET receptors, of which there are 2 types, ET_A and ET_B. Both receptors belong to the G-protein-coupled receptor (GPCR) system and mediate biological responses from a variety of stimuli, including growth factors, vasoactive polypeptides, neurotransmitters, hormones and phospholipids (1-3).

Endothelin-1 is produced by a wide variety of cells, including endothelial cells, vascular smooth muscle cells and various epithelial tissues (*e.g.*, bronchial, endometrial, mammary and prostatic) and is mitogenic for many cell types including endothelial cells, vascular and bronchial smooth muscle cells, fibroblasts, glomerular mesangial cells, osteoblasts, melanocytes and endometrial stromal cells. The mitogenic activity of ET-1 can be augmented by synergistic interactions with other growth factors including epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), insulin, insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), transforming growth factor (TGF) and interleukin-6 (IL-6) (4).

ET-1 is a relevant growth factor in many tumor types including carcinoma of the prostate, ovary, colon, cervix, breast, endometrium, as well as melanoma and Kaposi's sarcoma. ETs and their receptors have been implicated in cancer progression through autocrine and paracrine pathways (5-9). There is increasing evidence that implicates ET-1 in a range of disease processes involved in neoplasia, including cell proliferation, inhibition of apoptosis, matrix remodeling, bone deposition and metastases (Table I) (10). Identification of ET-1 as a mediator in the malignant progression of many tumors and the potential of the ET axis as a therapeutic target has propelled the investigation of ET-1 receptor antagonists into clinical trials (Table II) (5-36). Investigation into the role of the ET axis has accelerated markedly, with the development of several potent and selective ET-1 receptor antagonists. These molecules have provided tools for elucidating the biological significance of the ET axis and have strengthened the hypothesis that antagonism of the ET-1

Table I: Cancer-promoting functions of endothelins.

Proliferation	ET_A	Fibroblasts and smooth muscle cells Synergism with other growth factors Regulatory functions
	ET_B	Endothelial cells
Survival	ET_A	Fibroblasts and smooth muscle cells Cancer cells
	ET_B	Endothelial cells
Angiogenesis	ET_A	Proliferation of vascular smooth muscle cells and myofibroblasts Induction of VEGF
	ET_B	Proliferation and migration of endothelial cells
Metastasis	ET_A	Induction of MMPs Downregulation of TIMP-1 and TIMP-2 Induction of uPA and its receptor

system may have significant therapeutic potential (Table III) (37, 38).

Role of the endothelin axis in ovarian cancer

Signaling pathways activated by ET-1

ET-1, which is the most common circulating form of ETs, is produced by many epithelial tumors. The peptide signals through two G-protein-coupled receptors, ET_A and ET_B, that have different affinities for ETs (12). The ET_B receptor (ET_BR) binds the three peptide isotypes with equal affinity. In contrast, ET_AR binds ET-1 with higher affinity than the other isoforms. The ET-1/ET_AR autocrine pathway has a key role in the development and progression of prostatic, ovarian and cervical cancers.

We have previously demonstrated that ET-1 and the ET_AR are overexpressed in primary and metastatic ovarian carcinomas, as compared to normal ovaries. In ovarian tumor cells, ET-1 acts selectively as an autocrine growth factor through the ET_AR. Ligand binding to the receptor results in activation of a pertussis toxin-insensitive G-protein that stimulates phospholipase C activity and increases intracellular Ca^{2+} levels, activation of protein kinase C, MAPK and p125 focal adhesion kinase phosphorylation. Among downstream events after ET_AR activation in ovarian carcinoma, ET-1 causes epidermal growth factor (EGF) receptor transactivation, which is partly responsible for MAPK activation, suggesting that the coexistence of ET-1 and EGF autocrine circuits in these tumor cells could enhance their growth potential (39, 40).

ET-1 and tumor neovascularization

Endothelins, which function as mitogens for endothelial cells, vascular smooth muscle, fibroblasts and pericytes, are angiogenic factors. Endothelial cell mitogenesis is thought to be mediated by ET_BR, while vascular smooth muscle cells and pericyte mitogenesis appear to be mediated predominantly or solely by the ET_AR. ET-1 modulates various stages of neovascularization, including endothelial cell proliferation, migration, invasion, protease production and tube formation and stimulates neovascularization *in vivo* (41). Neovascularization is an early and critical event in ovarian cancer progression. In this regard, we have shown that elevated expression of ET-1 and its cognate receptor was significantly associated with microvessel density and vascular endothelial growth factor (VEGF) expression (13). Furthermore, activation of ET_AR by ET-1 stimulates VEGF production by increasing the levels of hypoxia-inducible factor-1alpha, a critical regulator of tumor growth and angiogenesis (42). These results indicate that ET-1 could modulate tumor angiogenesis through direct angiogenic effects on endothelial cells and, in part, through VEGF stimulation.

ET-1 and apoptosis

ET-1 acts as an antiapoptotic factor, suggesting that the peptide may also modulate cell survival pathways. This is further supported by the demonstration that ET-1 is effective in inhibiting paclitaxel-induced apoptosis, and that an ET_AR antagonist completely blocks the ET-1-induced survival effect. Engagement of the ET_AR by ET-1 triggers activation of antiapoptotic signaling through the Bcl-2-dependent and phosphatidylinositol 3-kinase-mediated Akt pathway (43).

ET-1 and tumor invasion

High levels of ET-1 were detected in the majority of ascitic fluids of ovarian cancer patients and were significantly correlated with VEGF ascitic concentrations, suggesting that ET-1 enhances the secretion of extracellular matrix-degrading proteinases (13). Thus, ET-1 acting through the ET_AR consistently induces the activity of two families of metastasis-related proteinases, matrix metalloproteinases (MMPs) and the urokinase type plasminogen activator system. Interestingly, we found that addition

Table II: Role of ET-1, its receptors and receptor antagonists in different malignancies.

Malignancy	Action of ET-1	ET receptors	Receptor antagonists and effects
Prostate cancer	Promotes prostate cancer growth, inhibits apoptosis through the ET_AR	High expression, decreased or absent ET_BR expression, frequent methylation of ET_AR gene	Atrasentan (ET_AR antagonist) relieved pain and delayed time to clinical and biochemical progression
Ovarian cancer	ET-1 mRNA was detected in 90%-100% of carcinomas examined. Promotes cell proliferation survival, invasion and VEGF-dependent angiogenesis through ET_AR	ET_AR mRNA was detected in 84% of carcinomas examined, ET_BR in only 40%. ET_AR mediated all ET-1 induced tumor promoting effects	BQ-123 (ET_AR antagonist) slowed the growth rate of tumor cells. Atrasentan inhibited growth of ovarian carcinoma xenografts and displayed additive effects in combination with taxanes
Melanoma	Promotes growth and migration of melanoma cells	ET_AR are downregulated in melanoma cells. ET_BR expression seems to be increased in melanoma cells in comparison to benign nevi	BQ-788 (ET_BR antagonist) inhibited growth of melanoma cell lines and reduced human melanoma tumor growth in nude mice. BQ-123 did not affect melanoma cell growth.
Bone malignancies	Increased the expression of osteocalcin protein and new bone formation. ET-1 overexpressing osteoblastic-inducing WISH tumor produced significantly more bone than control	Both ET_AR and ET_BR are expressed	BQ-123 blocked ET-1-induced osteocalcin expression. Atrasentan decreased ET-1-induced new bone formation and also inhibited osteoblastic bone metastases that occurred in prostate and breast cancer patients
Breast cancer	Increased ET-1 expression in cancer tissue, inversely correlated with the degree of tumor cell differentiation	Elevated expression of ET_AR has been detected in breast cancer tissue in comparison to normal tissue	
Renal cancer	ET-1 opposed the paclitaxel-induced apoptosis in renal carcinoma cell lines	All cell lines expressed ET_AR	
Lung cancer	ET-1 was detected in most squamous cell and adenocarcinomas	Both ET_AR and ET_BR are expressed. ET_AR seems downregulated in comparison to normal bronchial tissue	
Colon cancer	ET-1 protected colon carcinoma cells from FasL-induced apotosis	Increased expression of ET_AR and ET_BR in neoplastic tissue	Bosentan, mixed $ET_{A/B}R$ antagonist, potentiated FasL-induced apoptosis of tumor cells
Cervical cancer	ET-1 induced proliferation of HPV-positive cervical cell lines	Both ET_AR and ET_BR are expressed. Increased expression of ET_AR on HPV-positive cells	Atrasentan inhibited cell proliferation and the growth of cervical carcinoma xenografts and displayed additive effects in combination with taxanes
Kaposi's sarcoma	ET-1 and ET-3 induced cell proliferation, migration and invasion	Both ET_AR and ET_BR are expressed	$ET_{A/B}R$ antagonist blocked ET-1-induced cell proliferation and invasion and inhibited growth in nude mice
CNS tumors	ET-1 promoted meningioma cell proliferation	Both ET_AR and ET_BR are expressed	BQ-123 blocked ET-1-induced effects. ET_AR antagonist had no effect

of a specific ET_AR antagonist blocked ET-1-induced migration and invasion of ovarian carcinoma cells (44). Moreover, exposure of HEY and OVCA 433 ovarian carcinoma cell lines to ET-1 led to a 50-75% inhibition in gap junction intercellular communication (GJIC) and to a decrease in the connexin 43 (Cx43)-based gap junction plaques. To investigate the phosphorylation state of Cx43, ovarian carcinoma cell lysates were immunoprecipitated and transient tyrosine phosphorylation of Cx43 was detected in ET-1-treated cells. BQ-123, a selective ET_AR antagonist, blocked the ET-1-induced Cx43 phosphorylation and cellular uncoupling. Gap junction closure was prevented by tyrphostin 25 and by the selective c-Src inhibitor, PP2. Furthermore, the increased Cx43 tyrosine phosphorylation was correlated with ET-1-induced increase of c-Src activity and PP2 suppressed the ET-1-induced Cx43 tyrosine phosphorylation, indicating that inhibition of Cx43-based GJIC is mainly mediated by the

Table III: Therapeutic potential of ET_A antagonists in cancer.

Broad applicability to many cancer types
Special relevance for bone metastasis
Chemoprevention of cancer
Excellent safety profile
Potential to be combined with existing cytotoxic therapies, such as taxanes or radiation

Src tyrosine kinase pathway (45). These findings indicate that the signaling mechanisms involved in GJIC disruption on ovarian carcinoma cells depend on ET_AR activation which leads to the Cx43 tyrosine phosphorylation mediated by c-Src.

The ET_A receptor as a target for anticancer therapy

Ovarian cancer

In view of these findings, the ET_AR has been proposed as a potential target for anticancer therapy. The recent identification of low-molecular-weight compounds that inhibit ligand-induced activation of the ET_AR now offers the possibility of testing this therapeutic approach in a clinical setting. Among various ET_AR antagonists, ABT-627 (atrasentan) is an orally bioavailable endothelin antagonist that potently (K_i = 34 pM) and selectively binds to the ET_AR, blocking signal transduction pathways implicated in cancer cell proliferation and other host-dependent processes promoting cancer growth.

To evaluate the effect of ABT-627 on the proliferation of various ovarian carcinoma cells, we used two primary cultures (PMOV1 and PMOV2) and two established cell lines (HEY and OVCA 433). All of these cells express functional ET_AR and secrete high levels of ET-1. Primary cell cultures and cell lines were incubated for up to 5 days in the absence or presence of ABT-627 and ET_BR antagonists. In all ovarian carcinoma cells, spontaneous growth was significantly inhibited in the presence of ABT-627. Addition of the ET_BR antagonist did not affect the basal growth rate of the cells, demonstrating that endogenous ET-1 acts as an autocrine modulator of ovarian carcinoma cell proliferation only through ET_AR and can be selectively inhibited by ABT-627.

To determine whether the antiproliferative effect of ABT-627 resulted in the induction of programmed cell death, we measured the percentage of dying cells in ABT-627-treated and control cultures. ABT-627 treatment increased the percentage of apoptotic HEY and OVCA 433 ovarian cancer cells after 48 h of treatment. In these cells, activation of ET_AR by ET-1 prevents paclitaxel-induced apoptosis. We therefore evaluated the potential combined proapoptotic effect of treatment with an ET_AR antagonist and paclitaxel. HEY and OVCA 433 cells were incubated with paclitaxel alone or in combination with ABT-627. As expected, the addition of ABT-627 significantly increased paclitaxel-induced apoptosis in both cell lines. These results established that ET_AR-activated survival pathways can be affected by treatment with ABT-627. Therefore, ET_AR overexpression can promote tumor development through its stimulatory action on cancer cell growth and survival. However, ET_AR may also regulate angiogenesis by promoting tumor production of VEGF. Untreated HEY cells secreted approx. 375 pg VEGF/10^6 cells/24 h. Treatment with either ABT-627 or paclitaxel alone caused approx. 45% inhibition of VEGF secretion. Combination of ABT-627 with paclitaxel exerted a marked inhibitory effect, reaching almost 60% reduction of VEGF secretion to 150 pg/10^6 cells/24 h.

The potential antitumor effect of ABT-627 *in vivo* was assessed in murine tumor xenografts. Human HEY ovarian carcinoma cells were grown as subcutaneous tumors in nude mice. Seven days later, when well-established HEY xenografts were palpable with a tumor size of approx. 0.25 cm^3, mice were randomized into treatment and vehicle control groups of 10 animals each. The treated mice were injected i.p. for 21 days with two different concentrations of ABT-627, 2 mg/kg/day and 10 mg/kg/day. Treatment with ABT-627 produced a 65% inhibition of HEY tumor growth on day 40 after tumor injection with either low (2 mg/kg/day) or high (10 mg/kg/day) doses ($p < 0.001$ compared with control). ABT-627 treatment was generally well tolerated, with no detectable signs of acute or delayed toxicity even at the highest ABT-627 dose. Tumor growth suppression with 2 mg/kg/day ABT-627 was comparable to that achieved with paclitaxel (20 mg/kg i.v. x 3 every 4 days). Furthermore, the tumor growth inhibition obtained with ABT-627 persisted for up to 4 weeks following end of treatment. We then studied whether the proapoptotic effect of the combination of ABT-627 and paclitaxel that was observed *in vitro* could also be obtained *in vivo*. A more marked tumor growth inhibition (90% of controls) was observed with combination treatment with ABT-627 and paclitaxel, with no histological evidence of HEY tumors in 4 of 10 mice. The combination treatment at the dose and schedule tested was well tolerated, as judged by the absence of weight loss or other signs of acute or delayed toxicity. As compared with control tumor xenografts, the growth delay in established tumors lasted for up to 4 weeks following the end of treatment with ABT-627 and paclitaxel.

Because HEY cells express ET_AR and various autocrine and paracrine angiogenesis-related factors including ET-1, VEGF and MMP-2, we investigated the expression of these factors *in vivo* after ABT-627 treatment at a lower dose (2 mg/kg/day). Immunohistochemical analysis of the expression of VEGF, performed on HEY tumors on day 40 after tumor cell injection, revealed a marked reduction in the percentage of VEGF and MMP-2 positive HEY cells in ABT-627-treated mice. Tumor-induced vascularization, which was quantified as microvessel density (MVD) using antibody against CD31, was directly proportional to the expression of VEGF. There was a parallel reduction (45%) in MVD in tumors after treatment with ABT-627. A significant increase in

the percentage of TUNEL-positive cells was found in HEY tumors treated with ABT-627. The inhibition of human ovarian tumor growth in nude mice induced by ABT-627 was also associated with a reduction in Cx43 phosphorylation, suggesting that ET_AR blockade may also contribute to the control of ovarian carcinoma growth and progression by preventing the loss of GJIC (45).

Almost complete inhibition of VEGF, MMP-2 expression and tumor neovascularization, and an increase in apoptosis, were observed following combined treatment with ABT-627 and paclitaxel. HEY tumor xenografts freshly excised on day 40 after tumor cell injection were also analyzed for VEGF expression by Western blot. We observed a marked reduction of VEGF expression in animals treated with ABT-627 or paclitaxel. Approximately 70% inhibition was obtained when ABT-627 was combined with paclitaxel.

ET-1 acts through ET_AR as a survival factor by protecting HEY and OVCA 433 cells against paclitaxel-induced apoptosis via activation of antiapoptotic signaling pathways. Because impairment of apoptotic pathways is a major molecular mechanism leading to chemoresistance, ET_AR blockade by ABT-627 leading to sensitization of tumor cells to paclitaxel-induced apoptosis could produce an additive therapeutic effect. This indeed appears to occur *in vivo* following combined treatment with ABT-627 and paclitaxel, since the inhibition of tumor growth achieved with this protocol correlates with the highest tumor apoptotic index (46).

These findings demonstrate the antitumor activity of ABT-627 and provide a rationale for the clinical evaluation of this compound, alone and in combination with cytotoxic drugs, in patients with ovarian tumors and possibly in other epithelial tumors that overexpress functional ET_AR.

Prostate cancer

The ET axis has been identified as contributing to the pathophysiology of prostate cancer (7). In the normal prostate gland, ET-1 is produced by epithelial cells; the highest concentrations of ET-1 in the body are found in seminal fluid. In prostate cancer, key components of the ET-1 clearance pathway, ET_B and neutral endopeptidease, are diminished, resulting in an increase in local ET-1 concentrations. Increased ET_AR expression is also seen with advancing tumor stage and grade in both primary and metastatic prostate cancer. There are multiple pathways by which the ET-1/ET_A axis may promote prostate cancer progression. ET-1 is a mitogen for prostate cancer cell lines *in vitro* and acts synergistically with other peptide growth factors. ET-1 modulates apoptosis by affecting cell survival (6-9). ET-1 is also a mitogen for osteoblasts, the principal cell type in the osteoblastic response of bone in metastatic prostate cancer (47, 48).

Selective ET_AR antagonists may block the proliferative effects of exogenous ET-1 in both prostate cancer cells and osteoblasts. In a phase I clinical trial, patients with advanced prostate cancer were treated with atrasentan (ABT-627). In this phase I trial stabilization and decline of prostate-specific antigen (PSA) occurred in 66% of patients. In a phase II study, atrasentan suppressed markers of biochemical and clinical prostate cancer progression in bone (49). These data substantiate the role of the ET-1/ET_A axis as a growth and survival pathway and as a therapeutic target in hormone-refractory prostate cancer. Atrasentan may inhibit tumor growth in bone, both by direct effects on the tumor cells and by disrupting important bone/tumor interactions. In a randomized double-blind phase II study, 288 patients with hormone refractory prostate cancer were enrolled and treated with atrasentan (2.5 and 10 mg/day p.o.). In the 244 evaluable patients, atrasentan significantly delayed time to clinical and biochemical (PSA) progression. The most common side effects included rhinitis, peripheral edema and headache. Atrasentan also maintained total and bone alkaline phosphatase concentrations at baseline values compared to the placebo-treated group (50-52). These findings suggest that atrasentan may inhibit progression of hormone refractory prostate cancer in men with metastatic disease.

Cervical cancer

The ET-1/ET_AR autocrine also participates in the malignant progression of human papilloma virus-associated cervical carcinoma, in which ET_AR is increased and could be targeted for antitumor therapy. Thus, atrasentan inhibited the growth of cervical carcinoma cell xenografts. Two cycles of treatment completely reverted tumor growth. Atrasentan displayed additive effects when administrated in combination with the cytotoxic drug paclitaxel, supporting its clinical use as monotherapy or combination therapy (30, 31).

Kaposi's sarcoma

A different approach in targeting the ET-1 receptor in cancer treatment is given by Kaposi's sarcoma (KS), in which ET-1 acts as an autocrine growth factor via both ET_AR and ET_BR. Binding of ET-1 and ET-3 to both receptors increased the proliferation, migration and invasiveness of the KS-derived cell line, KS IMM cells, by stimulating secretion and activation of multiple tumor proteases. Therefore, we tested the small-molecule ET_A/ET_B antagonist A-182086 on KS tumor growth in nude mice. Treatment of mice with A-182086 resulted in tumor growth inhibition which was probably related to its antiproliferative effect on tumor cells and its antiangiogenic effect on endothelial cells expressing ET_BR. Thus, ET-1 receptor antagonists may be effective antiangiogenic and antitumor compounds for the treatment of KS (32, 33).

Conclusions

The ET axis represents a novel therapeutic target for cancer. ET-1 is overexpressed in many malignancies, acting as an autocrine growth factor. Engagement of the ET_AR by ET-1 triggers tumor proliferation, VEGF-induced angiogenesis, metastatic potential, antiapoptotic action and is synergistic with other growth factors. Direct mechanistic evidence of the role of ET_ARs in prostate, ovarian, cervical, colorectal and other cancers supports the concept that ET_AR antagonists will have a strong impact on the malignant phenotype (10). The antitumor activity of selective ET_AR antagonists observed in preclinical studies has been strongly supported in early clinical trials in which ET_AR antagonists have demonstrated a significant therapeutic potential (53).

Ongoing controlled studies will determine if promising early results will lead to a new generation of molecularly targeted therapies for cancer. Additional studies are warranted in combination with chemotherapy in ovarian cancer and other solid tumors expressing ET_AR.

Acknowledgements

I would like to thank L. Rosanò, F. Spinella and V. Di Castro for their contributions to studies on ovarian cancer cells, P.G. Natali for valuable comments on the article and P. Nisen (Abbott Laboratories, Global Oncology Development, Abbott Park, IL) for kindly providing atrasentan. This work was supported by the Associazione Italiana Ricerca sul Cancro and CNR-MIUR.

References

1. Levin, E.R. *Endothelins.* N Engl J Med 1995, 333: 356-63.

2. Goldie, R.G. *Endothelins in health and disease: An overview.* Clin Exp Pharmacol Physiol 1999, 26: 145-8.

3. Masaki, T. *The endothelin family: An overview.* J Cardiovasc Pharmacol 2000, 35: S3-S5.

4. Battistini, B., Chailler, P., D'Orleans-Juste, P., Briere, N., Sirois, P. *Growth regulatory properties of endothelins.* Peptides 1993, 14: 385-99.

5. Bagnato, A., Catt, K.J. *Endothelin as autocrine regulators of tumor cell growth.* Trends Endocrinol Metab 1998, 9: 378-83.

6. Nelson, J.B., Chan-Tack, K., Hedican, S.P. et al. *Endothelin-1 production and decreased endothelin B receptor expression in advanced prostate cancer.* Cancer Res 1996, 56: 663-8.

7. Nelson, J.B., Hedican, S.P., George, D.J. et al. *Identification of endothelin-1 in the pathophysiology of metastatic adenocarcinoma of the prostate.* Nat Med 1995, 1: 944-9.

8. Gohji, K., Kitazawa, S., Tamada, H., Katsuoka, Y., Nakajima, M. *Expression of endothelin receptor A associated with prostate cancer progression.* J Urol 2001, 165: 1033-6.

9. Nelson, J.B., Lee, W.H., Nguyen, S.H. et al. *Methylation of the 5' CpG island of the endothelin B receptor gene is common in human prostate cancer.* Cancer Res 1997, 57: 35-7.

10. Nelson, J.B., Bagnato, A., Battistini, B., Nisen, P. *The endothelin axis: Emerging role in cancer.* Nat Rev Cancer 2003, 3: 110-6.

11. Bagnato, A., Salani, D., Di Castro, V. et al. *Expression of endothelin 1 and endothelin A receptor in ovarian carcinoma: Evidence for an autocrine role in tumor growth.* Cancer Res 1999, 59: 720-7.

12. Moraitis, S., Langdon, S.P., Miller, W.R. *Endothelin expression and responsiveness in human ovarian carcinoma cell lines.* Eur J Cancer 1997, 33: 661-8.

13. Salani, D., Di Castro, V., Nicotra, M.R. et al. *Role of endothelin-1 in neovascularization of ovarian carcinoma.* Am J Pathol 2000, 157: 1537-47.

14. Moraitis, S., Miller, W.R., Smyth, J.F., Langdon, S.P. *Paracrine regulation of ovarian cancer by endothelin.* Eur J Cancer 1999, 35: 1381-7.

15. Bagnato, A., Tecce, R., Moretti, C., Di Castro, V., Spergel, D., Catt, K. *Autocrine actions of endothelin-1 as a growth factor in human ovarian carcinoma cells.* Clin Cancer Res 1995, 1: 1059-66.

16. Yohn, J.J., Smith, C., Stevens, T. et al. *Human melanoma cells express functional endothelin-1 receptors.* Biochem Biophys Res Commun 1994, 201: 449-57.

17. Kikuchi, K., Nakagawa, H., Kadono, T. et al. *Decreased ET_B receptor expression in human metastatic melanoma cells.* Biochem Biophys Res Commun 1996, 219: 734-9.

18. Eberle, J., Weitmann, S., Thieck, O., Pech, H., Paul, M., Orfanos, C.E. *Downregulation of endothelin B receptor in human melanoma cell lines parallel to differentiation genes.* J Invest Dermatol 1999, 112: 925-32.

19. Demunter, A., De Wolf-Peeters, C., Degreef, H., Stas, M., van den Oord, J.J. *Expression of the endothelin-B receptor in pigment cell lesions of the skin. Evidence for its role as tumor progression marker in malignant melanoma.* Virchows Arch 2001, 438: 485-91.

20. Lahav, R., Heffner, G., Patterson, P.H. *An endothelin receptor B antagonist inhibits growth and induces cell death in human melanoma cells in vitro and in vivo.* Proc Natl Acad Sci USA 1999, 96: 11496-500.

21. Nambi, P., Wu, H.L., Lipshutz, D., Prabhakar, U. *Identification and characterization of endothelin receptors on rat osteoblastic osteosarcoma cells: Down-regulation by 1,25-dihydroxy-vitamin D_3.* Mol Pharmacol 1995, 47: 266-71.

22. Yamashita, J., Ogawa, M., Inada, K., Yamashita, S., Matsuo, S., Takano, S. *A large amount of endothelin-1 is present in human breast cancer tissues.* Res Commun Chem Pathol Pharmacol 1991, 74: 363-9.

23. Alanen, K., Deng, D.X., Chakrabarti, S. *Augmented expression of endothelin-1, endothelin-3 and the endothelin-B receptor in breast carcinoma.* Histopathology 2000, 36: 161-7.

24. Giaid, A., Hamid, Q.A., Springall, D.R. et al. *Detection of endothelin immunoreactivity and mRNA in pulmonary tumours.* J Pathol 1990, 162: 15-22.

25. Thevarajah, S., Udan, M.S., Zheng, H., Pfluyg, B.R., Nelson, J.B. *Endothelin axis expression in renal cell carcinoma.* J Urol 1999, 161: 137-43.

26. Ahmed, S.I., Thompson, J., Coulson, J.M., Woll, P.J. *Studies on the expression of endothelin, its receptor subtypes, and*

converting enzymes in lung cancer and in human bronchial epithelium. Am J Respir Cell Mol Biol 2000, 22: 422-31.

27. Egidy, G., Juillerat-Jeanneret, L., Jeannin, J.F., Korth, P., Bosman, F.T., Pinet, F. *Modulation of human colon tumor-stromal interactions by the endothelin system.* Am J Pathol 2000, 157: 1863-74.

28. Eberl, L.P., Egidy, G., Pinet, F., Juillerat-Jeanneret, L. *Endothelin receptor blockade potentiates FasL-induced apoptosis in colon carcinoma cells via the protein kinase C-pathway.* J Cardiovasc Pharmacol 2000, 36: S354-6.

29. Eberl, L.P., Valdenaire, O., Saintgiorgio, V., Jeannin, J.F., Juillerat-Jeanneret, L. *Endothelin receptor blockade potentiates FasL-induced apoptosis in rat colon carcinoma cells.* Int J Cancer 2000, 86: 182-7.

30. Venuti, A., Salani, D., Manni, V., Poggiali, F., Bagnato, A. *Expression of endothelin-1 and endothelin A receptor in HPV-associated cervical carcinoma: New potential targets for anti-cancer therapy.* FASEB J 2000, 14: 2277-83.

31. Bagnato, A., Cirilli, A., Salani, D. et al. *Growth inhibition of cervix carcinoma cells in vivo by endothelin A receptor blockade.* Cancer Res 2002, 62: 6381-4.

32. Bagnato, A., Rosano, L., Di Castro, V. et al. *Endothelin receptor blockade inhibits proliferation of Kaposi's sarcoma cells.* Am J Pathol 2001, 158: 841-7.

33. Rosanò, L., Spinella, F., Di Castro, V. et al. *Endothelin receptor blockade inhibits molecular effectors of tumor invasion in Kaposi's sarcoma.* Am J Pathol 2003, 163: 753-62.

34. Pagotto, U., Arzberger, T., Hopfner, U. et al. *Expression and localization of endothelin-1 and endothelin receptors in human meningiomas. Evidence for a role in tumoral growth.* J Clin Invest 1995, 96: 2017-25.

35. Pagotto, U., Arzberger, T., Hopfner, U., Weindl, A., Stalla, G.K. *Cellular localization of endothelin receptor mRNAs (ET_A and ET_B) in brain tumors and normal human brain.* J Cardiovasc Pharmacol 1995, 26(Suppl. 3): S104-6.

36. Harland, S.P., Kuc, R.E., Pickard, J.D., Davenport, A.P. *Expression of endothelin$_A$ receptors in human gliomas and meningiomas, with high affinity for the selective antagonist PD156707.* Neurosurgery 1998, 43: 890-8.

37. Opgenorth, T.J. *Endothelin receptor antagonism.* Adv Pharmacol 1995, 33: 1-65.

38. Wu, C., Radford Decker, E., Holland, G.W. et al. *Nonpeptide endothelin antagonists in clinical development.* Drugs Today 2001, 37: 441-53.

39. Bagnato, A., Tecce, R., Di Castro, V., Catt, K.J. *Activation of mitogenic signaling by endothelin-1 in ovarian carcinoma cells.* Cancer Res 1997, 57: 1306-11.

40. Vacca, F., Bagnato, A., Catt, K.J., Tecce, R. *Transactivation of the epidermal growth factor receptor in endothelin-1-induced mitogenic signaling in human ovarian carcinoma cells.* Cancer Res 2000, 60: 5310-7.

41. Salani, D., Taraboletti, G., Rosano, L. et al. *Endothelin-1 induces an angiogenic phenotype in cultured endothelial cells and stimulates neovascularization in vivo.* Am J Pathol 2000, 157: 1703-11.

42. Spinella, F., Rosanò, L., Di Castro, V., Natali, P.G., Bagnato, A. *Endothelin-1 induces vascular endothelial growth factor by increasing hypoxia-inducible factor 1α in ovarian carcinoma cells.* J Biol Chem 2002, 277: 27850-5.

43. Del Bufalo, D., Di Castro, V., Biroccio, A. et al. *Endothelin-1 protects ovarian carcinoma cells against paclitaxel-induced apoptosis: Requirement for Akt activation.* Mol Pharmacol 2002, 61: 524-32.

44. Rosanò, L., Varmi, M., Salani, D. et al. *Endothelin-1 induces tumor proteinase activation and invasiveness of ovarian carcinoma cells.* Cancer Res 2001, 61: 8340-6.

45. Spinella, F., Rosanò, L., Di Castro, V., Nicotra, M.R., Natali, P.G., Bagnato, A. *Endothelin-1 decreases gap-junctional intercellular communication by inducing phosphorylation of connexin 43 in human ovarian carcinoma cells.* J Biol Chem 2003, 278: 41294-301.

46. Rosanò, L., Spinella, F., Salani, D. et al. *Therapeutic targeting of endothelin A receptor in human ovarian carcinoma.* Cancer Res 2003, 63: 2447-53.

47. Guise, T.A. *Molecular mechanisms of osteolytic bone metastases.* Cancer 2000, 88: 2892-8.

48. Chiao, J.W., Moonga, B.S., Yang, Y.M. et al. *Endothelin-1 from prostate cancer cells is enhanced by bone contact which blocks osteoclastic bone resorption.* Br J Cancer 2000, 83: 360-5.

49. Carducci, M.A., Nelson, J.B., Bowling, M.K. et al. *Atrasentan, an endothelin-receptor antagonist for refractory adenocarcinomas: Safety and pharmacokinetics.* J Clin Oncol 2002, 20: 2171-218.

50. Carducci, M.A., Padley, R.J., Breul, J. et al. *Effect of endothelin-A receptor blockade with atrasentan on tumor progression in men with hormone-refractory prostate cancer: A randomized, phase II, placebo-controlled trial.* J Clin Oncol 2003, 21: 679-89.

51. van der Boon, J. *New drug slows prostate-cancer progression.* Lancet Oncol 2002, 3: 201.

52. Yin, J.J., Mohammad, K.S., Kakonen, S.M. et al. *A causal role for endothelin-1 in the pathogenesis of osteoblastic bone metastases.* Proc Natl Acad Sci USA 2003.

53. Remuzzi, G., Perico, N., Benigni, A. *New therapeutics that antagonize endothelin: Promises and frustations.* Nat Rev Drug Discov 2002, 1: 986-1000.