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A review of the profile of endothelin axis in cancer and its management

Running head: endothelin in cancer

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Abstract

The endothelins and their associated receptors are important controllers of vascular growth, inflammation and vascular tone. In cancer, they have roles in the control of numerous factors in cancer development and progression, including angiogenesis, stromal reaction, epithelial mesenchymal transitions, apoptosis, invasion, metastases and drug resistance. Also, we consider current information on the role of this signalling system in cancer and examine the state of the current cell, animal and clinical trials utilising endothelin targeted drugs for cancer management. Although targeting the endothelin axis in cell lines and xenografts show some promise in retarding cellular growth, results from limited clinical trials in prostatic cancer are less encouraging and did not offer significant survival benefit. The ability to target both cancer cells and vasculature via endothelin is an important consideration that necessitates the further refining of therapeutic strategies as we continue to explore the possibilities of the endothelin axis in cancer treatment.

Keywords: endothelin; cancer; treatment; clinical trials

1. Introduction

Endothelins (ETs) are a family of genes, composed of sequences producing three 21 amino acid proteins, including endothelin-1 (ET-1), endothelin-2 (ET-2) and endothelin-3 (ET-3) (1). Endothelins derive from precursor proteins after cleavage by a specific metalloproteinase, endothelin converting enzyme (ECE) (2). Endothelin-1 (ET-1) is the predominant isoform in the family whereas isoforms ET-2 and ET-3 are less commonly expressed (1). ET-1 is also the most common circulating form of endothelin and is produced by somatic cells, including endothelial cells, vascular smooth muscle cells as well as many epithelial tumours. Endothelins are able to work in both paracrine and autocrine fashions, by binding to specific cell surface receptors, namely endothelin receptor A (ET_AR) and endothelin receptor B (ET_BR) (3). Binding of endothelins to their receptors mediates a number of functions, including vasoconstriction, pain, inflammation and most importantly, cancer (1, 2). The genes for ET-1, ET-2, ET-3, ET_AR and ET_BR are located on chromosomes 6, 1, 20, 4 and 13 respectively (4).

Of the two surface receptors for ET-1, ET_AR has more affinity for ET-1 and ET-2 than for ET-3, whereas ET_BR has equal affinity to all three ET isoforms. ET_AR is found on vascular smooth muscle cells while ET_BR is expressed on endothelial cells, vascular smooth muscle cells, macrophages and platelets (5). Also, both receptors are distributed in the kidney, airway smooth muscle cells and pulmonary vessels (6, 7).

In cancers, there are different expression profiles of the endothelin receptors when compared with normal tissues (Table 1) with ET_AR/ET_BR expression ratios differ significantly between cancer types. Regarding the expression level of endothelin receptors, cancers are divided into three categories : (1) cancers that express predominantly ET_AR such as nasopharyngeal, thyroid, prostate, colon, pancreatic, gastric, renal and breast cancers (8-15); (2) cancers predominately expressing ET_BR such as melanoma and brain tumours(

glioblastoma and astrocytoma) (16-18) and (3) cancers which express both ET_AR and ET_BR such as oral, lung, bladder, vulvar and ovarian cancers (19-23).

ET-1 mediates mitogenic effects in a variety of epithelial tumours via ET_AR (2) and in non-epithelial tumours such as melanoma by ET_BR (24). ET-1 is a mitogen for lymph vessel endothelial cells via ET_BR, so ET_BR blockade impairs lymphangiogenesis (25). Thus, ET_BR expression in cancer is associated with lymphatic invasion (26). Similarly, ET_AR overexpression in cancer has been shown to be associated with aggressive biological behaviour (27). This is reflected in the fact that high ET_AR expression is more prevalent in cancers with distant metastasis (8). The current review is to study the roles of endothelins in human cancer and the potential roles of them in cancer therapy.

2. The role of stroma in carcinogenesis related to endothelin

Cancer progression depends on the biological characteristics of the malignant cells and their interactions with benign cells and components of the surrounding stroma (28). Solid tumours are mixture of variety of cells; including cancer cells and cancer-associated cells (fibroblasts, macrophages and endothelial cells). All these cells can overexpress ET-1 and its receptors (29, 30).

Fibroblasts suppress preneoplastic epithelial proliferation (31). They are, however, the first cells that are recruited during early carcinogenesis and can be induced to secrete many factors that affect the cancer cells, which can result in increased aggressiveness (32). When they accumulate within the cancer area, they are termed cancer-associated fibroblasts (CAFs), becoming recruited by cancer signalling and have different morphological and functional features from their normal counterparts (33). CAFs have higher proliferative rates and are resistant to apoptosis (34). CAFs are made up of different cells from various sources and local stromal fibroblasts are the major source (35). Once recruited, fibroblasts may

differentiate to myofibroblasts. This process is called fibroblast to myofibroblast transdifferentiation, and is activated by several growth factors such as transforming growth factors-beta (TGF-ß), platelet-derived growth factor (PDGF), and basic fibroblast growth factor (bFGF) which are produced by cancer cells (36, 37). CAFs have an essential role in head and neck squamous cell carcinoma progression (38). The presence of myofibroblasts in the tumour stroma is a negative prognostic factor as they increase invasion of cancer cells. These myofibroblasts have a contractile phenotype and increase the proliferative and migratory capacity in cancer. For example, in oral squamous cell carcinoma (OSCC), the presence of myofibroblasts in the tumour stroma is associated with higher risk of invasion and poor prognosis (39). The behaviour of CAFs is also modulated in part by endothelins (40).

In the oral cavity, fibroblasts can amplify the proliferation and migration of cancer cells stimulated by ET-1. In this process, ET-1 stimulates the release of bioactive ligands such as ADAM17 from fibroblasts which bind to epidermal growth factor receptor (EGFR) on cancer cells and then trigger an increase in COX-2 expression (40). It was shown that in head and neck squamous cell carcinomas (HNSCC), the expression of COX-2 and EGFR correlated with a poor prognosis (41). These data suggest different mitogenic peptides may contribute to HNSCC progression and this could be evidence for stromal-epithelial interaction (41, 42). EGFR was also noted to be overexpressed in HNSCC patients in another study (43). Additionally, it was shown that activation of EGFR increases cancer cell migration and invasion due to decreasing cell adhesion (44).

ET-1 stimulates oral cancer cell invasion via both of its receptors, which are expressed by oral fibroblasts (40, 45). Oral fibroblast contractile phenotype is mediated primarily by ET_BR, however ET-1 stimulates oral fibroblasts to promote invasion of oral cancer cells in a paracrine manner (39).

3. Endothelin and angiogenesis in cancer

The tumour microenvironment is different from that of normal tissues. An important difference is that within a cancer, most regions are hypoxic (46). In addition, blood supply in cancer has a different structure from normal vessels (47). For example, vascular channels in cancer are often lined by cancer cells, mimicking a normal vessel, a state called "mimicry". Alternatively, vessels may be lined by cancer cells and endothelial cells, which is termed "mosaicism" (48, 49). Also, cancer blood vessels are highly permeable and prone to leaking (50). Cancers displaying vasculogenic mimicry proliferate rapidly and are more aggressive with higher risk of metastasis (51, 52). Hypoxia – inducible factor 1-α (HIF-1α) expression around the cancer cell-lined vessels is common and may show that hypoxia is involved in inducing cancer cell-lined vessel formation (53,54). HIF-1α also regulates vascular endothelial growth factor (VEGF) which has been indicated as an enhancer for formation of cancer cell lined vessels *in vitro* (54). In oral cancer, HIF-1α expression level was shown to be positively correlated with the aggressiveness of the cancer (55).

ET-1 activates the hypoxia-responsive pathway by inducing stabilization of HIF-1α under normoxic conditions. Then, HIF-1α up-regulates VEGF transcription. HIF-1α and VEGF production can thus be amplified by ET-1 under normoxic or hypoxic conditions (56). In cancer cells, ET-1 can itself be up-regulated due to hypoxia. This may lead to a positive feedback system, whereby ET-1 expression stimulated hypoxic pathways which stimulate further ET-1 expression, maintaining constant vascular expansion regardless of oxygen status. This dual role may explain the link between endothelins and cancer aggression.

4. Endothelin, EMT and metastasis

Epithelial mesenchymal transition (EMT), a cancer hallmark, involves losing epithelial markers such as E-cadherin, and gaining mesenchymal markers, like N-cadherin

and vimentin (57). The blockage of signals and pathways involving in EMT development is critical for reverting EMT and the related biological effects such as drug sensitivity. The ET-1/ ET_AR axis is one of these pathways, having several roles in cancer progression and its overexpression correlates with the advanced stages (29, 57, 58). As part of this association with advanced staging, the ET-1/ ET_AR axis has also been implicated in promotion of EMT as well as cell proliferation, angiogenesis, escape from apoptosis, invasion and metastasis (30). For example, it has been shown that overexpression of ET_AR in nasopharyngeal carcinoma initiates tumour cell metastasis (8).

Low E-cadherin and high vimentin in primary head and neck squamous cell carcinoma was associated with a 100% metastasis rate compared to those primary carcinomas with incomplete EMT markers, which were associated with a metastatic rate of 44% (59). Also, ET-1 and ET-3 have been found to up-regulate N-cadherin in human melanoma cell lines, a factor which is commonly associated with loss of E-cadherin (3). The observed down-regulation of E-cadherin and associated catenin adhesion proteins in melanocytes and melanoma cells are likely connected to the same mechanisms.

In addition to their roles in vasculogenesis, both VEGF and HIF-1α are associated with EMT in some cancers. For example, this phenomenon was observed in cancer cell lines from tongue and hypopharynx (60). VEGF is also correlated with invasion depth and increased risk of metastasis to lymph nodes in patients with head and neck squamous cell carcinoma (61). Due to its role in stimulating both VEGF production and HIF-1α stabilization, this places the endothelin axis at a significant control point for advanced tumour characteristics.

5. The ET-1 axis in chemo-resistance

Drug resistance is a major therapeutic barrier in cancer management. Several lines of evidence show an association between EMT phenotypes and chemoresistance in cancer cells (30). ET-1/ET_AR signalling has been demonstrated as having a key role in promoting EMT through regulating the interactions of cancer and microenvironment. ET-1/ET_AR knockdown, for example, has been shown to revert EMT phenotype, inhibit invasive behaviour, and increase the effects of chemotherapeutic agents (62). Therefore, it is suggested that ET_AR mediated EMT signalling in cancer cells occurs during resistance development. In support of this hypothesis, immunohistochemical analysis of human ovarian cancer tissues showed ET_AR overexpression in chemo-resistant cancers, suggesting ET_AR expression levels as a predictor of chemoresistance in cancer therapies (63). In addition, hypoxia has a critical role in acquisition of the EMT phenotype and chemoresistance onset. It is hypothesized that stimulation of the ET-1/ET_AR pathway may mimic a hypoxic environment via HIF-1α activation. On the other hand, ET-1 is itself targeted by HIF-1α, suggesting the presence of a feedback loop in the system. Together, these evidences suggest that the ET-1 axis contributes in signalling pathways triggering EMT, resulting in increased chemoresistance in cancer (63).

6. Endothelin and Cancer Therapeutics

Since endothelin mediated pathways contribute to cancer growth and progression, angiogenesis and metastasis in a variety of cancers, they are thus possible targets for cancer therapeutics. Several drugs are available, derived from both cancer research and the targeting of the endothelin axis for treatment of cardiovascular disease. The ET axis can be targeted by several approaches in cancer, such as endothelin-converting enzyme inhibition and antagonism of ET_A and ET_B receptors (58). Due to endothelin based effects on bone growth,

ET_AR antagonists have been suggested for the treatment of metastatic tumours in bone (64). Endothelin receptor antagonists can reduce angiogenesis by inhibiting endothelial cell mitogenesis. They might also have anti-apoptotic effects for endothelial cells by preventing the production of matrix metalloproteinases from macrophages (65). In normal cells, ET_BR regulates ET-1/ET_AR axis activities via mechanisms like increasing the production of nitric oxide, promoting ET-1 clearance, triggering apoptosis and blocking cell growth, but the same antagonistic effects are not proved to occur in tumour cells (66). In some cancers, such as breast cancer, ET_BR initiates invasion but in other cancers like prostate cancer, ET_BR absence results in ET-1 increasing due to reduced endothelin clearance (67, 68). Endothelin-converting enzyme is another potential therapeutic target, since it is required for the generation of active ET-1 peptides but a significant consideration for the use of this approach is that different isoforms of ECE-1 might have opposing effects (69).

The effects of endothelin antagonists in human cancer cell lines have been tested in multiple studies, and a summary of these can be seen in Table 2. The most commonly used antagonist in such studies is atrasentan. Atrasentan (ABT-627) is an orally bioavailable non-peptide, small molecule competitive ET inhibitor acting via decreasing the binding affinity of ET_AR for ET-1 (81). Atrasentan inhibited cell proliferation and increased apoptosis in nasopharyngeal cancer cell lines (8). In addition, atrasentan was shown to reduce cell invasion in breast cancer cell lines (70). In ovarian cell lines, atrasentan was found to increase apoptosis and inhibit cell proliferation and VEGF expression (71). Furthermore, it was found to lead to cell death in prostatic cancer (73).

The second most widely used ET inhibitor used in oncology trials is zibotentan (ZD4054), an orally bioavailable ET_AR antagonist (63, 74). It is the only ET antagonist which targets ET_AR without decreasing ET_BR expression level. Zibotentan has been found to inhibit cell proliferation and tumour growth in human ovarian carcinoma cell lines (7), but

not in breast cancer cell lines (75). In addition, zibotentan was found to revert the EMT phenotype, inhibit invasiveness and restore drug sensitivity in ovarian cancer cells (30). It reduces angiogenesis and also can reduce ET-1-induced epidermal growth factor receptor (EGFR) transactivation (76). Zibotentan combined with the anti-mitotic chemotherapy agents paclitaxel or docetaxel enhances apoptosis compared with either agent alone (77).

BQ123 is an ET_AR antagonist and BQ788 is selective ET_BR antagonist, which are often used in combination. Combination therapy with BQ123 and BQ788 were demonstrated to suppress growth of oral squamous cell carcinoma and oesophageal squamous cell carcinoma (78). In addition, these drugs in combination blocked the conversion of matrix metalloproteinases to active forms resulting in inhibition of cell invasiveness (79). On its own, BQ123 was found to inhibit the proliferation of colorectal and lung cancer cell lines (80, 81).

Bosentan is an endothelin receptor antagonist, which inhibits ET-1 action through a combination of interference with both ET_A and ET_B receptors (82). It has been found to induce cell death in human melanoma cell line (83).

These ET inhibitors have been shown to work on cancers in several animal xenograft models, the results of which are summarised in Table 3. In ovarian cancer, treatment with ET-1 antagonists such as atrasentan or zibotentan could inhibit 65% and 69% of the cancers in animals (7, 72). Mai *et al*. showed a 58% decrease in nasopharyngeal camcer volume by treating with Atrasentan (8). Also, Asham *et al*. noted a significant reduction in large bowel cancer load after treatment with the ET system antagonist, BQ123 (84). In breast cancer, Smollich *et al*. showed that zibotentan significantly decreased tumour volume (75). In addition, Rosano *et al.* had done an experience on complete inhibition of the proliferation of Kaposi's sarcoma by using A-182086, an $ET_A/_B$ receptor antagonist (79).

In some cancers, the combination of one endothelin receptor antagonist with an anticancer drug has shown good results as a useful therapeutic strategy. For example, ET_BR antagonism can improve the efficacy of paclitaxel on breast cancer in rats (7). In *in vitro* studies, using ET_AR antagonists such as zibotentan combining with paclitaxel or the DNA crosslinking chemotherapy agent cisplatinum showed an increase in antitumor activity in ovarian cancer (77, 85). ET-1 may modulate the resistance pathways during treatment with cytotoxic therapies via several mechanisms, though it is likely to be that abrogation of hypoxia is the main factor that can be blocked by ET_AR (86).

Despite the promising results in cell lines and animal studies, clinical trials results were disappointing. The bulk of clinical trials to date have taken place in prostate cancer. The 2 largest studies were based on 809 and 312 patients using atrasentan and zibotentan respectively (87, 88). In these studies, the treatment with endothelin antagonists showed no improvement in prognosis of patients with prostatic adenocarcinomas. A clinical trial was also performed in which bosentan was used to treat metastatic melanoma. The result of this trial was also disappointing, with only 6 of the 32 patients with the disease being stabilized by the treatment (89). Thus, more clinical trials may be needed for assessing the usefulness of the endothelin based treatments.

Endothelin receptors antagonists used in these trials have been noted to have some side effects. For example, liver toxicity was observed in 2-18% of the patients treated with bosentan (90). The most common side effects with atrasentan therapy were headache, rhinitis, and peripheral oedema (91).

7. Endothelins and bone formation

ET-1 stimulates new bone formation which can be blocked by ET_AR antagonists, but not ET_BR antagonists (92). In the late stage of prostate cancer and breast cancer, osteoblastic

activity is stimulated by factors secreted by cancer cells resulting in osteoblastic metastases, leading to the development of drugs targeting this process (93). A study on prostate cancer cell lines showed that the presence of both ET-1 and an anti-ET-1 antibody blocked osteoclastic bone resorption (94). Osteoblasts have a high affinity for ET_AR and ET-1 (95, 96). In animal studies, selective ET_AR antagonist treatment decreased new bone formation (97, 98). Additionally, atrasentan prolonged the time to the progression threshold levels of bone alkaline phosphatase; a biomarker of disease progression, by nearly twice compared with placebo in prostate cancer patients (505 days versus 254 days), though it did not reduce overall risk of progression (88).

8. Correlations with vascular endothelial growth factor

The current anti-angogenesis therapy focus on the action of vascular endothelial growth factor (VEGF). The prognostic and regulartory roles of VEGF have been extensviely studied (99-103). In fact, VEGF was shown to collaborate with ET-1 and ET_AR in cancer angiogenesis (104-106). Combination therapies targeting both VEGF and endothelins may be a potent control of angiogenesis.

9. Conclusion

In conclusion, while the ET system has plays an important role in the control of angiogenesis, it is not the only pathway that is involved in the control of angiogenesis. As demonstrated by the modest results of clinical trials to date, additional strategies may be needed to properly utilise this target for cancer treatment. Moreover, endothelin receptor antagonists may have different effects on different cells, depending on the interaction between the stroma and tumour cells in caner progression, along with the potential action of cancer stem cells. The mechanism of action in the clinical settings may be different from in

xenograft models. In xenograft model, due to shortened duration of cancer development, it is possible that interactions between cancer cells and stroma and cancer stem cells may not occur. This might explain the better endothelin antagonist effect on cell lines and xenografts than in clinical trials. The association between ET-1/ET_AR axis in both cancer progression and resistance to chemotherapy or radiotherapy, improves our understanding of the pathway and the mechanisms of drug resistance associated with it could be useful in cancer therapy. The combination of ET_AR antagonists with anti-cancer drugs has been shown to be relatively effective and can prevent EMT-related signalling. This allows both cancer cells and vasculature to be targeted. This is an important consideration, as both are involved in cancer progression. The ability of cancer cells to find alternative growth signalling pathways, also needs to be considered. Further refining of combined therapeutic strategies is necessary as we continue to explore the possibilities of the endothelin axis in cancer treatment.

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Alfred King-Yin Lam is Chair Professor and Head of Pathology who have published approximately 250 peer-reviewed articles in cancer research.

Table 1: Summary of ET receptors expression levels in different cancers.

Cancer type (Reference)	ET_AR expression	ET_BR expression
Nasopharyngeal cancer (8)	increased	decreased
Thyroid cancer (9)	increased	decreased
Prostate cancer (10)	increased	decreased
Colon cancer (11)	increased	decreased
Pancreatic cancer (12)	increased	decreased
Gastric cancer (13)	increased	decreased
Renal cancer (14)	increased	decreased
Breast cancer (15)	increased	decreased
Melanoma (16)	decreased	increased
Glioblastoma (17)	decreased	increased
Astrocytoma (18)	decreased	increased
Oral cancer (19)	increased	increased
Lung cancer (20)	increased	increased
Bladder cancer (21)	increased	increased
Vulvar cancer (22)	increased	increased
Ovarian cancer (23)	increased	increased

Table 2 Results of different types of ET antagonists in human cancer cell lines

Type of cell line (Reference)	Antagonist	Results
Nasopharyngeal carcinoma (8)	Atrasentan	growth inhibition by 38% and cell death
Breast adenocarcinoma (70)	Atrasentan	reduced cell invasion by 28%
Ovarian adenocarcinoma (71)	Atrasentan	significant increased apoptosis (15-17%)
Prostate adenocarcinoma (73)	Atrasentan	tumour cell death (18% - 60%)
Ovarian adenocarcinoma (7)	Zibotentan	significant increased apoptosis (13-39%)
Colorectal adenocarcinoma (80)	BQ123	decreased cell proliferation
Lung adenocarcinoma (81)	BQ123	decreased cell proliferation
Oral squamous cell carcinoma (19)	BQ123 and BQ788	decreased cell proliferation
Oral squamous cell carcinoma (78)	BQ123 and BQ788	suppression of cell growth
Oesophageal squamous cell carcinoma (78)	BQ123 and BQ788	suppression of cell growth
Kaposi's sarcoma (79)	BQ123 and BQ788	blocking the conversion of MMPs to active form
Melanoma (83)	Bosentan	induced cell death

Table 3: The effects of endothelin system antagonists on xenograft models

Type of cancer cell line	antagonist	Effects in the animals with cancer
Kaposi's sarcoma (79)	A-182086	Complete inhibition of cell growth
Ovarian carcinoma (72)	Atrasentan	65% inhibition of tumour growth
Nasopharyngeal carcinoma (8)	Atrasentan	58% decrease in tumour volume
Ovarian carcinoma (7)	Zibotentan	69% inhibition of tumour growth
Breast carcinoma (75)	Zibotentan	decreased tumour volume
Colon carcinoma (84)	BQ123	significant reduction in tumour load

References

1. Inoue A, Yanagisawa M, Kimura S, Kasuya Y, Miyauchi T, Goto K, et al. The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. Proc Natl Acad Sci USA. 1989;86:2863-7.

2. Grant K, Loizidou M, Taylor I. Endothelin-1: a multifunctional molecule in cancer. Br J Cancer. 2003;88:163-6.

3. Bagnato A, Natali PG. Endothelin receptors as novel targets in tumor therapy. J Transl Med. 2004;2:16.

4. http//www.ncbi.nlm.nlm.gov/gene.

5. Namiki A, Hirata Y, Fukazawa M, Ishikawa M, Moroi M, Aikawa J, et al. Endothelin-1- and endothelin-3-induced vasorelaxation via endothelium-derived nitric oxide. Jpn J Pharmacol. 1992;58 Suppl 2:326P.

6. Davenport AP, Morton AJ, Brown MJ. Localization of endothelin-1 (ET-1), ET-2, and ET-3, mouse VIC, and sarafotoxin S6b binding sites in mammalian heart and kidney. J Cardiovasc Pharmacol. 1991;17 Suppl 7:S152-5.

7. Rosano L, Di Castro V, Spinella F, Nicotra MR, Natali PG, Bagnato A. ZD4054, a specific antagonist of the endothelin A receptor, inhibits tumor growth and enhances paclitaxel activity in human ovarian carcinoma in vitro and in vivo. Mol Cancer Ther. 2007;6:2003-11.

8. Mai HQ, Zeng ZY, Feng KT, Ye YL, Zhang CQ, Liang WJ, et al. Therapeutic targeting of the endothelin a receptor in human nasopharyngeal carcinoma. Cancer Sci. 2006;97:1388-95.

9. Donckier JE, Michel L, Van Beneden R, Delos M, Havaux X. Increased expression of endothelin-1 and its mitogenic receptor ETA in human papillary thyroid carcinoma. Clin Endocrinol. 2003;59:354-60.

10. Godara G, Cannon GW, Cannon GM, Jr., Bies RR, Nelson JB, Pflug BR. Role of endothelin axis in progression to aggressive phenotype of prostate adenocarcinoma. Prostate. 2005;65:27-34.

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

12. Bhargava S, Stummeyer T, Hotz B, Hines OJ, Reber HA, Buhr HJ, et al. Selective inhibition of endothelin receptor A as an anti-angiogenic and anti-proliferative strategy for human pancreatic cancer. J Gastrointest Surg. 2005;9:703-9.

13. Mathieu MN, Chevillard C.Endothelin-1 and ETA receptor subtype are expressed in the gastric HGT-1 cell line. J Cardiovasc Pharmacol. 1995;26 Suppl 3:S508-9.

14. Pflug BR, Zheng H, Udan MS, D'Antonio JM, Marshall FF, Brooks JD, Nelson JB. Endothelin-1 promotes cell survival in renal cell carcinoma through the ET(A) receptor. Cancer Lett. 2007;246:139-48.

15.Hagemann T, Robinson SC, Schulz M, Trumper L, Balkwill FR, Binder C. Enhanced invasiveness of breast cancer cell lines upon co-cultivation with macrophages is due to TNF-alpha dependent up-regulation of matrix metalloproteases. Carcinogenesis. 2004;25:1543-9.

16. Demunter A, De Wolf-Peeters C, Degreef H, Stas M, van den Oord JJ. 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.

17. Paolillo M, Barbieri A, Zanassi P, Schinelli S. Expression of endothelins and their receptors in glioblastoma cell lines. J Neurooncol. 2006;79:1-7.

18. Shen C, Yang L, Yuan X.Endothelin B receptor expression in human astrocytoma: association with clinicopathological variables and survival outcomes. Int J Neruosci. 2001;1210:626-31.

19. Awano S, Dawson LA, Hunter AR, Turner AJ, Usmani BA. Endothelin system in oral squamous carcinoma cells: specific siRNA targeting of ECE-1 blocks cell proliferation. Int J Cancer. 2006;118:1645-52.

20. Ahmed SI, Thompson J, Coulson JM, Woll PJ. 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.

21. Wulfing C, Eltze E, Yamini J, Wulfing P, Bierer S, Bocker W, et al. Expression of the endothelin axis in bladder cancer: relationship to clinicopathologic parameters and long-term survival. Eur Urol. 2005;47:593-600.

22. Eltze E, Bertolin M, Korsching E, Wülfing P, Maggino T, Lellé R.Expression and prognostic relevance of endothelin-B receptor in vulvar cancer. Oncol Rep. 2007;18:305-11.

23. Bagnato A, Salani D, Di Castro V, Wu-Wong JR, Tecce R, Nicotra MR, 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.

24. Lahav R, Suva ML, Rimoldi D, Patterson PH, Stamenkovic I. Endothelin receptor B inhibition triggers apoptosis and enhances angiogenesis in melanomas. Cancer Res. 2004;64:8945-53.

25. Spinella F, Caprara V, Garrafa E, Di Castro V, Rosano L, Natali PG, et al. Endothelin axis induces metalloproteinase activation and invasiveness in human lymphatic endothelial cells. Can J Physiol Pharmacol. 2010;88:782-7.

26. Wulfing P, Diallo R, Kersting C, Wulfing C, Poremba C, Rody A, et al. Expression of endothelin-1, endothelin-A, and endothelin-B receptor in human breast cancer and correlation with long-term follow-up. Clinical Cancer Res. 2003;9:4125-31.

27. 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.

28. Deryugina EI, Quigley JP. Matrix metalloproteinases and tumor metastasis. Cancer Metastasis Rev. 2006;25:9-34.

29. Bagnato A, Rosano L. Epithelial-mesenchymal transition in ovarian cancer progression: a crucial role for the endothelin axis. Cells Tissues Organs. 2007;185:85-94.

30.Rosano L, Cianfrocca R, Spinella F, Di Castro V, Nicotra MR, Lucidi A, et al. Acquisition of chemoresistance and EMT phenotype is linked with activation of the endothelin A receptor pathway in ovarian carcinoma cells. Clinical Cancer Res. 2011;17:2350-60.

31. Schauer IG, Sood AK, Mok S, Liu J. Cancer-associated fibroblasts and their putative role in potentiating the initiation and development of epithelial ovarian cancer. Neoplasia. 2011;13:393-405.

32. Spano D, Zollo MTumor microenvironment: a main actor in the metastasis process. Clin Exp Metastasis. 2012; 29:381-95.

33. Orimo A, Gupta PB, Sgroi DC, Arenzana-Seisdedos F, Delaunay T, Naeem R, et al. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell. 2005; 121:335-48.

34. Kalluri R, Zeisberg M. Fibroblasts in cancer. Nat Rev Cancer. 2006;6:392-401.

35. Cirri P, Chiarugi P. Cancer-associated-fibroblasts and tumour cells: a diabolic liaison driving cancer progression. Cancer Metastasis Rev. 2012;31:195-208.

36. Untergasser G, Gander R, Lilg C, Lepperdinger G, Plas E, Berger P. Profiling molecular targets of TGF-beta1 in prostate fibroblast-to-myofibroblast transdifferentiation. Mech Ageing Dev. 2005;126:59-69.

37. Zhang J, Liu J. Tumor stroma as targets for cancer therapy. Pharmacol Ther. 2013;137: 200-15.

38. Lim KP, Cirillo N, Hassona Y, Wei W, Thurlow JK, Cheong SC, et al. Fibroblast gene expression profile reflects the stage of tumour progression in oral squamous cell carcinoma. J Pathol. 2011;223:459-69.

39. Marsh D, Suchak K, Moutasim KA, Vallath S, Hopper C, Jerjes W, et al. Stromal features are predictive of disease mortality in oral cancer patients. J Pathol. 2011; 223: 470-81.

40. Hinsley EE, Hunt S, Hunter KD, Whawell SA, Lambert DW. Endothelin-1 stimulates motility of head and neck squamous carcinoma cells by promoting stromal-epithelial interactions. Int J Cancer. 2012;130:40-7

41. Kalyankrishna S, Grandis JR. Epidermal growth factor receptor biology in head and neck cancer. J Clin Oncol. 2006;24:2666-72.

42. Quang PN, Schmidt BL. Endothelin-A receptor antagonism attenuates carcinoma-induced pain through opioids in mice. J Pain. 2010;11:663-71.

43. Thariat J, Etienne-Grimaldi MC, Grall D, Bensadoun RJ, Cayre A, Penault-Llorca F, et al. Epidermal growth factor receptor protein detection in head and neck cancer patients: a many-faceted picture. Clin Cancer Res. 2012;18:1313-22.

44. Barr S, Thomson S, Buck E, Russo S, Petti F, Sujka-Kwok I, et al. Bypassing cellular EGF receptor dependence through epithelial-to-mesenchymal-like transitions. Clin Exp Metastasis. 2008;25:685-93.

45.Hinsley EE, Kumar S, Hunter KD, Whawell SA, Lambert DW. Endothelin-1 stimulates oral fibroblasts to promote oral cancer invasion. Life Sci. 2012; 91:557-61

46. Höckel M, Vaupel P.Tumor hypoxia: definitions and current clinical, biologic, and molecular aspects. J Natl Cancer Inst. 2001;93:266-76.

47. Nagy JA, Chang SH, Shih SC, Dvorak AM, Dvorak HF. Heterogeneity of the tumor vasculature. Semin Thromb Hemost. 2010; 36:321-31.

48. Maniotis AJ, Folberg R, Hess A, Seftor EA, Gardner LM, Pe'er J, et al. Vascular channel formation by human melanoma cells in vivo and in vitro: vasculogenic mimicry. Am J Pathol. 1999;155:739-52.

49. Chang YS, di Tomaso E, McDonald DM, Jones R, Jain RK, Munn LL. Mosaic blood vessels in tumors: frequency of cancer cells in contact with flowing blood. Proc Natl Acad Sci U S A. 2000;97:14608-13.

50. Hobbs SK, Monsky WL, Yuan F, Roberts WG, Griffith L, Torchilin VP, et al. Regulation of transport pathways in tumor vessels: role of tumor type and microenvironment. Proc. Natl Acad. Sci. USA.1998;95: 4607–4612.

51. Seftor EA, Meltzer PS, Kirschmann DA, Pe'er J, Maniotis AJ, Trent JM, et al. Molecular determinants of human uveal melanoma invasion and metastasis. Clin Exp Metastasis. 2002; 19:233-46.

52. Paulis YW, Soetekouw PM, Verheul HM, Tjan-Heijnen VC, Griffioen AW. Signalling pathways in vasculogenic mimicry. Biochim Biophys Acta. 2010;1806:18-28.

53. van der Schaft DW, Hillen F, Pauwels P, Kirschmann DA, Castermans K, Egbrink MG, et al. Tumor cell plasticity in Ewing sarcoma, an alternative circulatory system stimulated by hypoxia. Cancer Res. 2005; 65:11520-8.

54. Rybak SM, Sanovich E, Hollingshead MG, Borgel SD, Newton DL, Melillo G, et al. “Vasocrine" formation of tumor cell-lined vascular spaces: implications for rational design of antiangiogenic therapies. Cancer Res. 2003; 63:2812-9.

55. Zhang Q, Zhang ZF, Rao JY, Sato JD, Brown J, Messadi DV, et al. Treatment with siRNA and antisense oligonucleotides targeted to HIF-alpha induced apoptosis in human tongue squamous cell carcinomas. Int J Cancer. 2004;111:849-57.

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

57. Rosano L, Spinella F, Di Castro V, Nicotra MR, Dedhar S, de Herreros AG, et al. Endothelin-1 promotes epithelial-to-mesenchymal transition in human ovarian cancer cells. Cancer Res. 2005;65:11649-57.

58. Kedzierski RM, Yanagisawa M. Endothelin system: the double-edged sword in health and disease. Annu Rev Pharmacol Toxicol. 2001;41:851-76.

59. Nijkamp MM, Span PN, Hoogsteen IJ, van der Kogel AJ, Kaanders JH, Bussink J. Expression of E-cadherin and vimentin correlates with metastasis formation in head and neck squamous cell carcinoma patients. Radiother Oncol. 2011; 99:344-8.

60. Yang MH, Wu MZ, Chiou SH, Chen PM, Chang SY, Liu CJ, et al. Direct regulation of TWIST by HIF-1alpha promotes metastasis. Nat Cell Biol. 2008; 10:295-305

61. Kim SH, Cho NH, Kim K, Lee JS, Koo BS, Kim JH, Chang JH, Choi EC. Correlations of oral tongue cancer invasion with matrix metalloproteinases (MMPs) and vascular endothelial growth factor (VEGF) expression. J Surg Oncol. 2006; 93:330-7.

62. Polyak K, Weinberg RA. Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. Nat Rev Cancer. 2009; 9:265-73.

63. Rosano L, Spinella F, Bagnato A. The importance of endothelin axis in initiation, progression, and therapy of ovarian cancer. Am J Physiol Regul Integr Comp Physiol. 2010;299:R395-404.

64. Lalich M, McNeel DG, Wilding G, Liu G. Endothelin receptor antagonists in cancer therapy. Cancer Invest. 2007;25:785-94.

65. Grimshaw MJ. Endothelins and hypoxia-inducible factor in cancer. Endocr Relat Cancer. 2007;14:233-44.

66. Lambert LA, Whyteside AR, Turner AJ, Usmani BA. Isoforms of endothelin-converting enzyme-1 (ECE-1) have opposing effects on prostate cancer cell invasion. Br J Cancer. 2008;99:1114-20.

67. Grimshaw MJ, Hagemann T, Ayhan A, Gillett CE, Binder C, Balkwill FR. A role for endothelin-2 and its receptors in breast tumor cell invasion. Cancer Res. 2004;64:2461-8.

68.Nelson JB, Lee WH, Nguyen SH, Jarrard DF, Brooks JD, Magnuson SR, 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.

69. Opgenorth TJ, Adler AL, Calzadilla SV, Chiou WJ, Dayton BD, Dixon DB, et al. Pharmacological characterization of A-127722: an orally active and highly potent ETA-selective receptor antagonist. J Pharmacol Exp Ther.1996;276:473-81.

70. Smollich M, Gotte M, Kersting C, Fischgrabe J, Kiesel L, Wulfing P. Selective ETAR antagonist atrasentan inhibits hypoxia-induced breast cancer cell invasion. Breast cancer Res Trea 2008;108:175-82.

71. Rosano L, Spinella F, Di Castro V, Natali PG, Bagnato A. Therapeutic targeting of the endothelin-A receptor in human ovarian carcinoma: efficacy of cytotoxic agents is markedly enhanced by co-administration with atrasentan. J Cardiovasc Pharmacol 2004;44:S132-5.

72. Rosano L, Spinella F, Salani D, Di Castro V, Venuti A, Nicotra MR, et al. Therapeutic targeting of the endothelin a receptor in human ovarian carcinoma. Cancer Res 2003;63:2447-53.

73. Banerjee S, Hussain M, Wang Z, Saliganan A, Che M, Bonfil D, et al. In vitro and in vivo molecular evidence for better therapeutic efficacy of ABT-627 and taxotere combination in prostate cancer. Cancer Res 2007;67:3818-26.

74. Morris CD, Rose A, Curwen J, Hughes AM, Wison DJ, Webb DJ. Specific inhibition of the endothelin A receptor with ZD4054: clinical and pre-clinical evidence. Br J Cancer. 2005;92:2148–2152.

75. Smollich M, Gotte M, Fischgrabe J, Macedo LF, Brodie A, Chen S, et al. ETAR antagonist ZD4054 exhibits additive effects with aromatase inhibitors and fulvestrant in breast cancer therapy, and improves in vivo efficacy of anastrozole. Breast Cancer Res Treat. 2010;123:345-57.

76. Rosano L, Di Castro V, Spinella F, Tortora G, Nicotra MR, Natali PG, et al. Combined targeting of endothelin A receptor and epidermal growth factor receptor in ovarian cancer shows enhanced antitumor activity. Cancer Res. 2007;67:6351-9.

77. Growcott JW. Preclinical anticancer activity of the specific endothelin A receptor antagonist ZD4054. Anticancer Drugs 2009;20:83-8.

78. Ishimoto S, Wada K, Tanaka N, Yamanishi T, Ishihama K, Aikawa T, et al. Role of endothelin receptor signalling in squamous cell carcinoma. Int J Oncol. 2012; 40:1011-9.

79. Rosanò L, Spinella F, Di Castro V, Nicotra MR, Albini A, Natali PG, et al. Endothelin receptor blockade inhibits molecular effectors of Kaposi's sarcoma cell invasion and tumor growth in vivo. Am J Pathol. 2003;163:753-62.

80. Grant K, Knowles J, Dawas K, Burnstock G, Taylor I, Loizidou M. Mechanisms of endothelin 1-stimulated proliferation in colorectal cancer cell lines. Br J Surg. 2007;94:106-12.

81. Zhang WM, Zhou J, Ye QJ. Endothelin-1 enhances proliferation of lung cancer cells by increasing intracellular free Ca2+. Life Sci. 2008;82:764-71.

82. Clozel M, Breu V, Gray GA, Kalina B, Löffler B-M, Burri K, et al. Pharmacological characterization of bosentan, a new potent orally active nonpeptide endothelin receptor antagonist. J Pharmacol Exp Ther. 1994;270:228–235.

83. Berger Y, Bernasconi CC, Juillerat-Jeanneret L. Targeting the endothelin axis in human melanoma: combination of endothelin receptor antagonism and alkylating agents. Exp Biol Med. 2006;231:1111-9.

84. Asham E, Shankar A, Loizidou M, Fredericks S, Miller K, Boulos PB, et al. Increased endothelin-1 in colorectal cancer and reduction of tumour growth by ET(A) receptor antagonism. Br J Cancer. 2001;85:1759-63.

85. Jiao WJ, Xu J, Pan H, Wang TY, Shen Y. Effect of endothelin-1 in esophageal squamous cell carcinoma invasion and its correlation with cathepsin B. World J Gastroenterol. 2007;13:4002-5.

86. Smollich M, Gotte M, Kersting C, Fischgrabe J, Kiesel L, Wulfing P. Selective ETAR antagonist atrasentan inhibits hypoxia-induced breast cancer cell invasion. Breast cancer Res Trea 2008;108:175-82.

87. James ND, Caty A, Payne H, Borre M, Zonnenberg BA, Beuzeboc P, et al. Final safety and efficacy analysis of the specific endothelin A receptor antagonist zibotentan (ZD4054) in patients with metastatic castration-resistant prostate cancer and bone metastases who were pain-free or mildly symptomatic for pain: a double-blind, placebo-controlled, randomized Phase II trial. BJU Int. 2010;106:966-73.

88. Carducci MA, Saad F, Abrahamsson PA, Dearnaley DP, Schulman CC, North SA, et al. A phase 3 randomized controlled trial of the efficacy and safety of atrasentan in men with metastatic hormone-refractory prostate cancer. Cancer 2007;110:1959-66.

89. Kefford R, Beith JM, Van Hazel GA, Millward M, Trotter JM, Wyld DK, et al. A phase II study of bosentan, a dual endothelin receptor antagonist, as monotherapy in patients with stage IV metastatic melanoma. Invest New Drugs. 2007;25:247-52.

90. Fattinger K, Funk C, Pantze M, Weber C, Reichen J, Stieger B, et al. The endothelin antagonist bosentan inhibits the canalicular bile salt export pump: a potential mechanism for hepatic adverse reactions. Clin Pharmacol Ther. 2001;69:223-31.

91. Carducci MA, Padley RJ, Breul J, Vogelzang NJ, Zonnenberg BA, Daliani DD, 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.

92. Alam AS, Gallagher A, Shankar V, Ghatei MA, Datta HK, Huang CL, et al. Endothelin inhibits osteoclastic bone resorption by a direct effect on cell motility: implications for the vascular control of bone resorption. Endocrinology 1992;130:3617-24.

93. Fox E, Curt GA, Balis FM. Clinical trial design for target-based therapy. Oncologist. 2002;7:401-9.

94. Chiao JW, Moonga BS, Yang YM, Kancherla R, Mittelman A, Wu-Wong JR, 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.

95. Spinella F, Rosano L, Di Castro V, Nicotra MR, Natali PG, 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

96.Sampaio AL, Rae GA, Henriques MG. Effects of endothelin ETA receptor antagonism on granulocyte and lymphocyte accumulation in LPS-induced inflammation. J Leukoc Biol. 2004;76:210-6.

97. Guise TA, Yin JJ, Mohammad KS. Role of endothelin-1 in osteoblastic bone metastases. Cancer. 2003;97:779-84.

98.Nelson JB, Nguyen SH, Wu-Wong JR, Opgenorth TJ, Dixon DB, Chung LW, et al. New bone formation in an osteoblastic tumor model is increased by endothelin-1 overexpression and decreased by endothelin A receptor blockade. Urology. 1999;53:1063-9.

99. Salajegheh A, Smith RA, Kasem K, Gopalan V, Nassiri MR, William R, Lam AK. Single nucleotide polymorphisms and mRNA expression of VEGF-A in papillary thyroid carcinoma: potential markers for aggressive phenotypes. Eur J Surg Oncol. 2011; 37: 93-9.

100. Yu XM, Lo CY, Lam AK, Lang BH, Leung P, Luk JM. The potential clinical relevance of serum vascular endothelial growth factor (VEGF) and VEGF-C inrecurrent papillary thyroid carcinoma. Surgery. 2008;144: 934-40;

101. Yu XM, Lo CY, Lam AK, Leung P, Luk JM. Serum vascular endothelial growth factor C correlates with lymph node metastases and high-risk tumor profiles in papillary thyroid carcinoma. Ann Surg. 2008; 247: 483-9.

102. Yu XM, Lo CY, Chan WF, Lam KY, Leung P, Luk JM. Increased expression of vascular endothelial growth factor C in papillary thyroid carcinoma correlates with cervical lymph node metastases. Clin Cancer Res. 2005; 11: 8063-9.

103. Salajegheh A, Pakneshan S, Rahman A, Dolan-Evans E, Zhang S, Kwong E, et al. Co-regulatory potential of VEGF-A and VEGF-C in thyroid carcinoma. Hum Pathol 2013 (in press).

104. Boldrini L, Gisfredi S, Ursino S, Faviana P, Lucchi M, Melfi F, et al. Expression of endothelin-1 is related to poor prognosis in non-small cell lung carcinoma. Eur J Cancer 2005; 41:2828-35.

105. Jiao W, Xu J, Zheng J, Shen Y, Lin L, Li J. Elevation of circulating big endothelin-1: an independent prognostic factor for tumor recurrence and survival in patients with esophageal squamous cell carcinoma. BMC Cancer. 2008; 8:334.

106. Wülfing P, Kersting C, Tio J, Sonntag B, Buerger H, Wülfing C, et al. Endothelin-1-, endothelin-A-, and endothelin-B-receptor expression is correlated with vascular endothelial

growth factor expression and angiogenesis in breast cancer. Clin Cancer Res.2004; 10:2393-400.