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Endothelins and their Receptors in Cancer: Identification of Therapeutic Targets

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Abstract

Endothelins and their receptors are important in normal physiology, but have been implicated in various pathophysiological conditions. Members of the so-called “endothelin axis” are dysregulated in a wide range of human cancers, opening the door for novel anticancer therapies. Established cancer chemotherapeutic agents and drugs that target specific components of the endothelin axis have been combined with promising results, but more work is needed in this area. The endothelin axis affects numerous signaling pathways, including Ras, mitogen activated protein kinases, β -catenin/T-cell factor/lymphoid enhancer factor, nuclear factor- κ B (NF κ B), SNAIL, and mammalian target of rapamycin (mTOR). There is much still to learn about optimizing drug specificity in this area, while minimizing off-target effects. Selective agonists and antagonists of endothelins, their receptors, and upstream processing enzymes, as well as knockdown strategies *in vitro*, are providing valuable leads for testing in the clinical setting. The endothelin axis continues to be an attractive avenue of scientific endeavor, both in the cancer arena and for other important health-related disciplines.

Keywords

Cancer therapeutics; endothelin axis; ET-1; endothelin-converting enzyme; invasion/metastasis; immune function

1. Introduction

Endothelins (ETs) are small peptides that interact with G-protein-coupled receptors and have important roles in biology and disease development [1–8]. Interest in this area is illustrated by the fact that a PubMed search using the term “endothelin” identified 23,714 separate items, over 1,000 of which encompassed “endothelins and cancer”. The latter topic has been covered by several excellent reviews, including those of Bagnato and Rosano [8], Bhalla *et al.* [9], Kandalaf *et al.* [10], and Lalic *et al.* [11]. The present review seeks to provide recent updates on the endothelin field as it pertains to cancer etiology, and a perspective on the most promising areas for therapeutic intervention in cancer patients.

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2. Endothelins, receptors, and G-protein signaling

The ET peptides ET-1, ET-2 and ET-3 are encoded by distinct genes, but the three final biologically-active products all have 21-amino acids, an α -helical structure, and two disulfide bonds. ET-2 and ET-3 differ by 2 and 6 amino acids, respectively, from ET-1 [1]. In the case of ET-1, an initial 212-amino acid prepro-ET-1 product is cleaved by endothelin converting enzyme (ECE-1) to generate big-ET-1 containing 38 amino acids, and further cleavage generates a C-terminal fragment along with the active peptide (half-life ~1 minute in the circulation). Clearance involves either catabolism via the neutral endopeptidase neprilysin, or lysosomal degradation in response to receptor-mediated uptake. Hypoxia, shear stress, growth factors and cytokines can stimulate ET production, whereas prostacyclin, nitric oxide (NO), atrial natriuretic peptide, and certain dietary phytochemicals act in an inhibitory fashion.

Two quite distinct surface receptors mediate cellular responses to ETs. ET_AR has high affinity for ET-1 and ET-2 but low affinity for ET-3, whereas all three ETs have similar affinity for ET_BR. The two receptor subtypes both contain seven transmembrane domains, but differences in their C-terminal sequences affect G-protein coupling, resulting in divergent intracellular responses following ligand-mediated activation at the surface. Notably, intracellular signaling triggered by ET_BR-ligand binding typically operates in a counter-regulatory fashion to ET_AR activation, and *vice versa*. Major pathways and effectors downstream of ET receptors include mitogen activated protein kinases (MAPK), adenylyl cyclase, phospholipases, and various immediate early genes [12]. Thus, rather than a linear response, a complex network of signaling pathways relays the activation signal from the cell surface to the nucleus (Figure 1). For example, interleukin-6, epidermal growth factor, insulin-like growth factor, transforming growth factor, and basic fibroblast growth factor play an integral part in the mitogenic response to ET-1 [13].

Cross-talk also occurs with other cell surface receptors, including the epidermal growth factor receptor (EGFR), leading to MAPK activation and the involvement of c-Src [14]. Combining ET_AR antagonists with EGFR inhibitors is a logical approach, especially in the treatment of ovarian cancer, as demonstrated in preclinical models [8]. This also may be feasible in the treatment of ET_AR-overexpressing non-small cell lung cancer, where monotherapy with an EGFR inhibitor gefinitib has the same efficacy as routine combination chemotherapy. In the latter case, the addition of an ET_AR antagonist holds some promise for improved clinical efficacy over gefinitib alone [9,15].

3. Endothelins and cell survival

In various cancer cell types, ET-1 inhibits apoptosis via the modulation of key survival pathways. For example, through alterations in the phosphorylation status of Bcl-2, ET-1 attenuated paclitaxel-induced apoptosis in ovarian carcinoma lines, and this was blocked by selective ET receptor antagonists [16]. The important implication from these and other studies on cancer cell survival is that ET receptor antagonists, acting on Bcl-2 family members, might help to reverse drug resistance and augment conventional chemotherapy [17,18]. This topic was reviewed recently by Bagnato and Rosano [8].

4. Endothelins and neovascularization

In addition to mitogenic actions on endothelial cells, fibroblasts, and vascular smooth muscle cells, ETs serve as angiogenic factors. Neovascularization stages that are impacted by ET-1 include protease production, tube formation, endothelial cell proliferation, migration, and invasion. Microvessel density and vascular endothelial growth factor levels are positively associated with ET-1 expression, and this can be amplified under conditions

of hypoxia. ET-1 potentiates hypoxia signaling via regulation of hypoxic inducible factor-1 α (HIF-1 α). Indeed, a reciprocal relationship has been proposed in which ET-1 stabilizes HIF-1 α resulting in the activation of HIF-1 α -regulated angiogenic genes, including HIF-1 α -mediated transcription of ET-1 itself. Thus, ET expression can be influenced by the tumor microenvironment, and ETs then modify that environment through the actions of HIF-1 α [19]. These interactions are generally amplified under conditions of hypoxia as compared with normoxic conditions.

Under normoxic conditions, ET-1 enhances cyclooxygenase (COX)-1 and COX-2 expression and prostaglandin E₂ (PGE₂) levels. Inhibitors of COX enzymes interfere with ET-1-induced vascular endothelial growth factor (VEGF) and PGE₂ production, matrix metalloproteinase (MMP) activity, and cell invasion [20]. These effects were blocked by chemical inhibitors or siRNA-mediated silencing of ET-1 receptor signaling, and knockdown of HIF-1 α desensitized cells at the level of COX-2 transcription, MMP activation, PGE₂ and VEGF production. Under normoxic or hypoxic conditions, HIF-1 α and ET cross-talk thus serves to augment various steps in tumor progression and invasion [8,20]. A recent study concluded that through regulation of the HIF-prolyl hydroxylase domain 2 and concomitant HIF-1 α stabilization, ET-1 regulates angiogenesis and cell invasion in melanoma cells [21].

5. Endothelins and tumor invasion/metastasis

ET-1/ET_AR interactions affect key players in metastasis, such as MMPs and the urokinase type plasminogen activator system [22]. There is increased expression of endothelins and their receptors in invasive breast cancer, resulting in cross-talk with cytokines, MMPs, and tumor-associated macrophages [23]. ET-1 stimulates lymphatic vessels and lymphatic endothelial cells to grow and invade [24]. In ovarian cancer cells, ET_AR, β -arrestin and β -catenin interact to induce cell invasion and metastasis [25]. Interestingly, stromal endothelin B receptor-deficiency inhibits growth and metastasis in breast cancer cells [26]. Endothelin receptor antagonists are among the arsenal of therapeutic approaches for metastatic castration-resistant prostate cancer [27], as well as metastatic bladder cancer [28]. Cueni *et al.* [29] observed that a membrane glycoprotein, podoplanin, increased tumor lymphangiogenesis and metastasis to regional lymph nodes *in vivo*. Transcriptional profiling of tumor xenografts identified a potential role for ET-1, changes in the expression of which were correlated with lymph node metastasis and reduced survival times in a cohort of 252 oral squamous cell carcinoma patients [29]. In the case of glioma, extracranial metastasis is rare due to the lack of lymphatic drainage in the brain coupled with poor penetration into blood vessels; thus, ET_BR antagonists might act locally to block cancer cell proliferation and induce apoptosis [30].

6. Endothelins and intercellular communication

Tumor progression has been associated with dysregulated intercellular communication and altered levels of connexin (Cx) proteins [31]. Earlier findings indicated that ET-1 and angiotensin-II increased gap junctional conductance between cardiomyocytes, and MAPK inhibition revealed that extracellular signal-regulated kinases (ERK) 1/2 were critical for up-regulation of Cx43 in response to ET-1 [32]. However, ETs were reported to act as potent inhibitors of gap junctional communication in hippocampal slices [33], and in cortical astrocytes low nM concentrations of either ET-1 or ET-3 produced robust inhibition of Cx43 expression [34]. In human ovarian cancer cells, ET-1 decreased gap junctional communication by inducing phosphorylation of Cx43 [35]. Cx43 expression also was implicated in the actions of ET-1 on human osteoblastic cell differentiation, suggesting that Cx43/ET-1 play a role in the response of osteoblasts to mitogenic factors in bone

pathologies, including cancer [36,37]. For further information on the role of endothelins in bone remodeling, which may have particular relevance in prostate and breast cancers, the reader is referred to published reviews [38,39].

7. Endothelins and immune modulation

The activation of tumor-infiltrating immune cells, and their differentiation and trafficking, may be regulated by ETs in some circumstances [19]. ET receptors are present on tumor-associated macrophages, which not only respond to ETs but also produce them. No such activity was detected in cell extracts from lymphocytes and neutrophils. Interestingly, ET_BR-specific blockade increases T-cell homing to tumors and augments the efficacy of immunotherapy [10].

8. ET axis and cancer therapeutics

Bagnato *et al.* [39] reviewed the “endothelin axis” and the diverse range of human cancer types examined to date. Specifically, ETs and their receptors have been implicated in cancers of the ovary, prostate, cervix, breast, lung, bladder, colon, nasopharynx, and endometrium, as well as in melanoma, neuroblastoma, osteosarcoma and Kaposi’s sarcoma. Some of these cancer sites were alluded to above, and the reader is referred for further information to [39] and the synopsis shown in Table 1. Studies in colorectal cancer are noteworthy because in addition to altered endothelin receptor subtypes [40], direct transcription of the ET-1 gene (*EDN1*) by β -catenin has been reported [41], along with the diagnostic potential of ET-1 in colon cancer patients [42]. The authors’ laboratory focuses on dietary chemopreventive agents that inhibit colorectal cancer, including epigallocatechin-3-gallate (EGCG) and other tea catechins [43–45]. Tea polyphenols are of interest because they block receptor tyrosine kinase activity and invasiveness in colon cancer cells [45,46], and have been shown to inhibit the endothelin axis and downstream signaling in ovarian cancer cells [47]. Red wine polyphenols, such as resveratrol, have been implicated in lowering ET-1 levels [48,49], and the soy isoflavone genistein restored endothelial function in chicks via changes in NO and ET-1 [50].

As attractive as these candidates are from dietary sources, one concern is that they can exhibit pleiotropic effects. Thus, research has focused on targeted therapy towards individual members of the endothelial axis, with the goal of improving efficacy compared with existing standards of care. Specific and non-specific ET_A and ET_B antagonists, as well as ECE inhibitors may be of value; however, to date, interruption of the ET-axis has met with mixed levels of success. For example, the earlier encouraging results that were obtained in the clinical investigation of an orally bioavailable ET_A-selective antagonist, atrasentan, in prostate cancer patients [51] were not sustained in phase 3 clinical trials with the agent in the same setting [52]. The promising delay in disease progression did not translate into an overall-survival benefit. However, despite these disappointing results, atrasentan in combination with docetaxel may provide an alternate treatment option in this disease setting [53]. The specific ET_A antagonist, zibotentan, also has been evaluated in the same patient settings that were used for the atrasentan clinical trials. More encouragingly, the phase 2 clinical trial that evaluated safety and efficacy of zibotentan in patients with metastatic castration-resistant prostate cancer (CRPC) did provide an overall survival benefit compared with placebo [54]. Consistent with these findings, the final analysis showed overall survival/hazard ratios of less than one had been sustained for zibotentan [55]. A large phase 3 clinical trial program is further evaluating the therapeutic potential of zibotentan in men with CRPC. Pre-clinical data with ET_A-antagonists also provide a strong rationale for potential clinical evaluation in other tumors. Zibotentan produced additive effects when combined with aromatase inhibitors and fulvestrant in pre-clinical models of breast cancer [56], and both

zibotentan and atrasentan have shown efficacious outcomes in pre-clinical models of ovarian cancer (reviewed in [8]).

Furthermore, with the approval of the ET_A-selective antagonist ambrisentan for use in pulmonary arterial hypertension, there has been increased support to clinically test this type of agent in other settings unrelated to the primary indication, where block of ET_A may turn out to be of benefit. One example might be in the treatment of metastatic ovarian cancer, particularly as an adjunct following debulking surgery [57].

A randomized, double-blind, placebo-controlled trial of bosentan, an ET_AR/ET_BR dual antagonist, was performed in patients with stage IV metastatic melanoma [58]. No effect was seen with respect to time to tumor progression in patients receiving decarbazine as first-line chemotherapy. There is clinical evidence that bosentan might prove effective in patients with neuroendocrine tumors and presenting with carcinoid heart disease, based on serological, echocardiographic, and clinical markers [59].

ET_BR selective antagonists also are undergoing preclinical evaluation, such as BQ788 [10]. It remains to be determined whether ET_B-selective agents will prove to be clinically effective for certain cancer subtypes, as distinct from those targeted by ET_A antagonists.

Further upstream, ECE has been considered as a potential therapeutic target, since it is required for generation of the biologically active ET-1 peptide. In ovarian cancer cells, silencing of ECE-1 reduced ET-1-dependent p44/42 MAPK phosphorylation, decreased invasiveness and MMP2 activity, improved adhesion to basal lamina proteins, laminin-1, and collagen IV, and increased E-cadherin while reducing N-cadherin expression [60]. However, one potential complication is that different isoforms of ECE-1 might have opposing effects [61]; in matrigel assays, overexpression of ECE-1c augmented PC-3 prostate cancer cell invasion, whereas ECE-1a was suppressive. ECE-1a expression in stromal cells also counteracted the effects of ECE-1c in PC-3 cells. It remains to be determined whether unique differences in ECE-1 isoform expression occur in other cancer types, which could open the avenue for selective targeting of ECE-1 isoforms for each malignancy. Of related interest, recent studies showed that ECE-1 inhibition enhanced substance P-induced expression and phosphorylation of the nuclear death receptor Nur77, resulting in cell death [62]. Agonist availability in endosomes, regulated by ECE-1, was observed to control β -arrestin-dependent signaling of endocytosed G protein-coupled receptors. Chemical screening of ECE antagonists has identified several interesting leads, including CGS35066 [63], SM19712 [64], RO67-7447 [65], and various indole-based compounds [66] with nM IC₅₀ values. Kirkby *et al.* [67] provided interesting insights into the various challenges that are encountered with ECE inhibition, and the pros and cons of specific ECE inhibitors tested to date.

9. Conclusions

Endothelins and their receptors are dysregulated in a host of human cancers. Accumulating evidence supports the view that individual members of the endothelin axis represent novel targets for anticancer therapy. A promising approach involves combined treatment modalities, in which the efficacy of well established chemotherapeutic agents is enhanced by targeting specific components of the endothelin axis. Because the endothelin axis itself impacts upon numerous signaling pathways, there is much still to learn about optimal approaches to minimize potential off-target effects. Highly selective antagonists targeting either ET_AR or ET_BR, as well as ECE inhibitors, may prove useful in the clinical setting. The endothelin axis remains an interesting and active avenue of scientific endeavor, both in the cancer area and in other important pathological conditions [68].

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Figure 1. Endothelins, their receptors, and downstream signaling pathways

Figure modified from previous schemes [1,8,10,19], with updates as indicated in the text. For abbreviations see text and [8]. Examples are shown at the top of the figure of cancer therapeutic/chemopreventive agents that have been examined for their effects on the endothelin axis. The figure is over-simplified and does not detail the full extent of cross-talk among the various intracellular signaling pathways. Nor does it accurately reflect the complete range of actions for the inhibitors shown. For example, EGCG acts on Met and other tyrosine kinases, as well as on downstream components of NFκB and β-catenin/T-cell factor/lymphoid enhancer (β-cat/Tcf/Lef) factor signaling.

Table 1

Endothelins and their receptors are implicated in diverse cancer types

Cancer type	Key findings and selected clinical observations	Refs
Bladder	VEGF/EGFR pathways are therapeutic targets for metastatic bladder cancer; improved with other therapies including aurora kinase inhibitors, endothelin receptor antagonists, RAS/MAPK pathway inhibitors, novel immunologic strategies.	[28]
Bone	Safety/efficacy of ET _A R antagonist zibotentan (ZD4054) studied in patients with metastatic castration-resistant prostate cancer and bone metastases.	[54] [55]
Breast	ET _A R correlates with aggressive carcinoma; MMP/cytokine cross-talk. ET _A R antagonist ZD4054 exhibits additive effects with aromatase inhibitors and fulvestrant in breast cancer therapy, and improves <i>in vivo</i> efficacy of anastrozole.	[23] [26] [56]
Cervical	ET _A R is functional in HPV-positive carcinoma.	[38]
Colorectal	ET-1 is a transcriptional target of β -catenin/Tcf/Lef; clinical studies of VEGF/ET interactions are currently in progress.	[40] [41]
Glioblastoma	ET _B R antagonists block proliferation and induce apoptosis in glioma cells.	[30]
Kaposi's sarcoma	ET _A R/ET _B R readily detected; dual antagonists inhibit xenografts.	[38]
Lung	ET _A R antagonists combined with EGFR show promise in the treatment of patients with non-small-cell lung cancer.	[8] [9] [15]
Melanoma	ET _B R is over-expressed; ET-1 inhibits prolyl hydroxylase domain 2 to activate HIF-1 α in melanoma cells.	[21] [38]
Nasopharyngeal	Three-quarters of such tumors overexpress ET _A R.	[38]
Neuroblastoma	Cells express ECE-1; BQ123 blocks proliferation <i>in vitro</i> ; statins induce Bcl-2 via ET-1 and NFATc3 in SH-SY5Y cells.	[19]
Osteosarcoma	ET _A R is expressed; ET-1 promotes MMP induction via NF κ B.	[19] [38]
Ovarian	ET _A R is present in primary and metastatic cancers; zibotentan + cisplatin + paclitaxel is effective for epithelial ovarian cancer.	[16] [17] [18]
Prostate	Zibotentan dose escalation study noted prolonged stable disease in some patients; results supported ET _A R antagonism as a viable approach; phase 3 trial under way.	[27] [54] [55]
Renal	ET _A R is expressed in some renal cancer lines.	[27]