Endothelin 1 in cancer: biological implications and therapeutic opportunities

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Abstract | Activation of autocrine and paracrine signalling by endothelin 1 (ET1) binding to its receptors elicits pleiotropic effects on tumour cells and on the host microenvironment. This activation modulates cell proliferation, apoptosis, migration, epithelial-to-mesenchymal transition, chemoresistance and neovascularization, thus providing a strong rationale for targeting ET1 receptors in cancer. In this Review, we discuss the advances in our understanding of the diverse biological roles of ET1 in cancer and describe the latest preclinical and clinical progress that has been made using small-molecule antagonists of ET1 receptors that inhibit ET1-driven signalling.

Nociceptive
Pertaining to nociceptors, which are nerves with specialized receptors that send pain signals to the brain and spinal cord.

Autocrine
A mode of signalling in which a secreted substance acts on surface receptors that are present on the same cell from which the substance was produced.

Paracrine
A form of bioregulation in which a secretion produced by one cell type in a tissue diffuses through the tissue and affects another cell type in the same tissue.

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Aberrant activation of the endothelin 1 (ET1) axis — which consists of the ligand ET1 and its receptors, endothelin A receptor (ETAR) and ETBR — is now recognized as a common mechanism underlying the progression of various solid tumours, including ovarian, prostate, colon, breast, bladder and lung cancers[1–3]. This activation can be initiated by the overexpression of ET1, by the loss of a negative regulator or by deregulated expression of the ET1 receptors or scaffold molecules. ET1 signalling is influenced by the spatial and temporal context of ET1 receptor activation, as well as by the formation of signalling complexes that interconnect with other signalling pathways. As a result, the ET1 axis can activate proliferation, confer apoptosis resistance, stimulate new vessel formation, modulate immune responses, induce abnormal osteogenesis, alter nociceptive stimuli, and promote invasion and metastatic dissemination. As the cellular activity of ET1 depends on contextual cues, in this Review, we outline our current understanding of the relevance of ET1 autocrine and paracrine signalling to cancer progression through a network of cellular pathways and interactions with the tumour microenvironment. Moreover, we address the therapeutic implications of these findings, particularly the blockade of ET1 receptors using small molecules, and their potential for combination with classic chemotherapy or other targeted agents. Improved knowledge of the complexity of ET1-triggered signalling and the identification of predictive markers will allow the full exploitation of these innovative therapeutic strategies.

The ET1 signalling network

Endothelin synthesis and regulation. The endothelins comprise a family of three endogenous, vasoactive, 21-amino-acid-long peptides, ET1, ET2 and ET3, which are characterized by a single α-helix and two disulphide bridges at Cys3–Cys11 and Cys1–Cys15 (REF. 4). In this Review, we focus on the best-characterized isoform, ET1; for further information on ET2 and ET3 (see REF. 5) (BOX 1).

The endothelins are encoded by distinct genes (endothelin 1 (*EDN1*), *EDN2* and *EDN3*). Human *EDN1* encodes the ET1 precursor pre-pro-ET1 and is located on chromosome 6, spanning 6.8 kb and containing five exons. *EDN1* is thought to be regulated primarily at the level of transcription, which is induced by more than 20 different stimuli, each acting in certain cells and tissues and each regulated by different transcription factors[6]. *EDN1* is under the transcriptional control of a TATA box-containing promoter. Within the proximal promoter, a functional activator protein 1 site has been identified, which can be bound by the FOS and JUN transcription factors. This promotes the transcription of *EDN1* in response to various stimuli, including phorbol esters, thrombin, angiotensin II and insulin, which signal through different pathways, including protein kinase C (PKC) and JUN N-terminal kinase (JNK) pathways[6]. Other regulatory elements in the proximal *EDN1* promoter include binding sites for GATA family transcription factors, transforming growth factor-β (TGFβ)-activated SMAD transcription factors and hypoxia-inducible factor 1 (HIF1).

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Key points

- Aberrant expression of endothelin 1 (ET1), or overexpression of endothelin receptors or their linked signalling circuits can contribute to tumour initiation and progression through both autocrine and paracrine mechanisms. These alteration mechanisms may arise from genetic and epigenetic changes.
- An intricate network of crosstalk between ET1 signalling and other growth factor pathways drives tumour progression. This includes crosstalk between the endothelin receptors and epidermal growth factor receptor and vascular endothelial growth factor receptor.
- ET1 signalling promotes cell proliferation, survival, epithelial-to-mesenchymal transition, neovascularization, response of immune cells and drug resistance in a context-dependent manner. Hence, endothelin receptors have emerged as key targets for cancer therapy.
- In addition to tumour cells, endothelin receptors are found on tumour-associated host cells, such as blood and lymphatic endothelial cells, fibroblasts and inflammatory cells, thus regulating the contribution of these cell types to cancer progression. Therefore, endothelin receptor antagonists may inhibit tumour progression by blocking crucial signalling events in both the tumour microenvironment and the tumour cells.
- The activation of ET1 signalling pathways is often negatively correlated with patient outcomes in different types of cancer.
- Small-molecule antagonists for targeting endothelin receptors have been evaluated in several recent clinical trials. However, the clinical results to date have been disappointing and it is crucial to decipher why the promising preclinical data have not yet been translated to the clinic.
- Future improved clinical trials might incorporate predictive biomarkers to focus on subsets of patients who are most likely to respond, use other clinical settings or use rational combination therapy with chemotherapeutics or targeted agents.

This HIF1 binding site is crucial for the response to hypoxia and growth factors, including ET1 (discussed below). In the distal *EDN1* promoter, several regulatory elements have been identified. These include hormone-response elements (which are activated by steroid hormones or mineralocorticoids), a nuclear factor-κB (NF-κB) binding site (which is activated by glucose, tumour necrosis factor-α (TNFα), interferon-γ, interleukin-1β (IL-1β) and other cytokines), an E-box motif and two nuclear factor of activated T cells binding sites[6]. Furthermore, emerging evidence indicates that epigenetic mechanisms, such as DNA methylation and histone modifications, are important for *EDN1* regulation, as observed in breast cancer cells with bone tropism[6,7]. Recently, it was found that ET1 expression is also regulated by post-transcriptional modifications that affect mRNA stability. The 3′-untranslated region (3′ UTR) of *EDN1* mRNA contains AUUUA motifs, which recruit proteins that specifically bind AU-rich elements to regulate RNA degradation or stabilization[6]. Additionally, *EDN1* mRNA is also subject to microRNA (miRNA)-mediated regulation[6]: in HeLa and hepatocarcinoma cells, it was found that ET1 expression is regulated through the binding of miR-1 to the 3′ UTR of *EDN1* mRNA[8,9].

The primary translation product of the *EDN1* gene is the 212-amino-acid-long pre-pro-ET1. This is cleaved by an endopeptidase to form the 38-amino-acid-long, biologically inactive precursor known as big-ET1, which is then further cleaved into ET1 by endothelin converting enzyme 1 (ECE1), or, alternatively, by a chymase enzyme[6]. Furthermore, two pathways have been described for the clearance of ET1: endothelin–ETBR-mediated internalization followed by lysosomal degradation, and removal by extracellular neutral endopeptidase (NEP; also known as endopeptidase 24.11)[6].

β-arrestins
A family of proteins that interact with the carboxyl termini of G protein-coupled receptors and that help to mediate receptor desensitization, internalization, recycling and signalling.

Endothelin receptor activation. ETAR and ETBR are distinct G protein-coupled receptors (GPCRs) of the family A (the 'druggable' class) of GPCRs. ETBR has equal affinities for all three endothelins, whereas ETAR exhibits an affinity for ET1 and ET2 that is two orders of magnitude higher than that for ET3. Endothelin receptors in cancer cells can be activated either through autocrine production of ligand or through production of ligand from stromal cells that may be expressed physiologically or in response to cancer cells in a paracrine loop[1,3,10]. Both receptors activate a similar signalling cascade, resulting in a range of pleiotropic responses (FIG. 1).

Crosstalk with other signalling pathways. ET1 signalling triggers the activation of a diverse network of other signalling pathways. These include MAPK, PI3K–AKT, NF-κB, β-catenin, HIF1α, RHO, phospholipases, PKA and PKC[1]. Further detail on these pathways (FIGS 1,2) is described below in the context of the biological roles of these pathways in cancer.

Some noteworthy crosstalk is the ET1-mediated transactivation of receptor tyrosine kinases (RTKs) and β-catenin signalling. β-arrestins 1 and 2 are scaffold proteins that bind to GPCRs such as ETAR and ETBR and that serve as molecular 'hubs' to organize complex signalling networks[11,12]. This leads, via the intracellular tyrosine kinase SRC, to the transactivation of RTKs such as epidermal growth factor receptor (EGFR) and vascular endothelial growth factor receptor 3 (VEGFR3)[13–17]. An alternative route by which ET1 activates EGFR is through the activation of the protease ADAM17, which causes the cleavage and ectodomain shedding of TNFα and other EGFR ligands to trigger EGFR signalling[18].

Multiple mechanisms involving β-arrestin 1 have been reported that allow interactions between ET1 and β-catenin signalling in tumour cells[16,19–21]. Interaction between activated ET1 receptors and β-arrestin 1 at the cell membrane can result in a physical association either with axis inhibition 1 (AXIN1), which contributes to the release and inactivation of glycogen synthase kinase 3β (GSK3β) and β-catenin stabilization, or with SRC to transactivate EGFR and lead to β-catenin tyrosine phosphorylation and activation[16] (FIG. 2a). Additionally, β-arrestin–β-catenin interactions can occur in the nucleus[21]. In this recently identified mechanism, ETAR engagement by ET1 results in the nuclear translocation of β-arrestin 1, where it directly binds to β-catenin to enhance its nuclear accumulation and transcriptional activity. β-arrestin 1–β-catenin interactions activate β-catenin target genes — such as *EDN1*, *AXIN2*, matrix metalloproteinase 2 (*MMP2*) and cyclin D1 (*CCND1*) — by promoting the dissociation of histone deacetylase 1

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and the recruitment of the p300 acetyltransferase to the promoters of these genes, resulting in enhanced histone H3 and H4 acetylation[21]. Transcriptional activation of *EDN1* by β-catenin has been observed in colon, prostate and ovarian cancer cell lines[19–21] and creates a self-amplifying positive-feedback loop that forms an ET1 autocrine circuit (FIG. 2a).

Roles of ET1 in cancer cell biology

Cell proliferation. Although different cancer cell types vary dramatically in their growth responses to stimulation by ET1, it is clear that autocrine ET1 signalling has a crucial role in tumour growth and survival. Early studies in various tumour cells, including prostate, cervical and ovarian cancer cells, revealed that spontaneous growth was significantly inhibited by ETAR antagonists, such as atrasentan (ABT-627) and zibotentan (ZD4054), demonstrating that endogenous ET1 acts as an autocrine modulator of cell proliferation through ETAR[22–25]. As discussed above, ET1 signalling has crosstalk with various well-characterized signalling pathways that act in a synergistic and combinatorial manner to convey mitogenic signals to the nucleus and promote cell proliferation. Furthermore, the mitogenic activity of ET1 can be amplified by synergistic interactions with other growth factors, including EGF, basic fibroblast growth factor, insulin, insulin-like growth factor, platelet-derived growth factor, TGFβ and IL-6 (REF. 1).

Cell survival. Inhibition of apoptosis is an essential step in tumorigenesis. ET1 is an anti-apoptotic factor in different cell types and acts by modulating cell survival pathways, such as PI3K-dependent AKT activation or NF-κB signalling[25–27]. In particular, ETAR blockade by atrasentan in prostate cancer cells induces the inactivation of NF-κB DNA-binding activity and a subsequent decrease in the levels of the anti-apoptotic proteins BCL-2, BCL-X_L and survivin, which leads to apoptotic cell death[25]. In ovarian cancer cells, ET1 markedly inhibited paclitaxel-induced apoptosis, as a result of phosphorylation of the anti-apoptotic protein BCL-2, and the apoptotic response could be restored by treatment with the specific ETAR antagonist BQ-123 (REF. 26). Thus, ETAR blockade may result in antitumour activity by growth inhibition and by

Box 1 | **ET2 and ET3**

Overexpression of endothelin 1 (ET1) has been documented in various human cancers, but much less is known about the roles of ET2 and ET3. ET2 and ET3 are encoded by the genes endothelin 2 (*EDN2*) and *EDN3*, respectively. ET2 differs from ET1 by only two amino acids, and, unlike the third isoform, ET3, it has the same affinity as ET1 for both endothelin A receptor (ETAR) and ETBR[111]. It was often assumed that ET2 would mimic the actions of the more abundant ET1. Growing interest in ET2 in cancer pathogenesis has begun after reports of *EDN2* overexpression in human breast cancer, especially in hypoxic areas[112,113]. In addition to regulating cancer cell apoptosis, ET2 has also been shown to affect invasive and metastatic potential[113]. Furthermore, hypoxic areas expressing ET2 attract and activate macrophages. In the presence of activated macrophages, breast tumour cells migrated towards ET1 and ET2 but not towards ET3, and they invaded tissues and metastasized distally[5,114]. Elevated ET2 levels have also been detected in basal cell carcinoma, characterized by aberrant activation of Hedgehog (HH) signalling. Moreover, the 3′ promoter region of *EDN2* contains the GLI-binding site, indicating that *EDN2* can be a direct target gene of HH signalling[115]. Recently, a role for ET2 in melanocyte stem cell behaviour has been reported[116]. This study identified *EDN2* as a direct target of nuclear factor 1 B-type (NFIB), a coordinator of the crosstalk between epithelial and melanocyte stem cells within a hair follicle niche to sustain hair regeneration and pigmentation. ET2, but not ET1 or ET3, can influence melanocyte stem cell proliferation and differentiation, an effect that is prevented by BQ-788 (REF. 116). These findings emphasize the importance of ET2 as a relevant messenger to uncouple melanocyte and hair follicle stem cell synchrony.

In humans, ET3 differs from ET1 by six amino acids and is the only isoform that has differential affinities for ETAR and ETBR, as it is highly selective for ETBR. In ETBR- or ET3-knockout mice, the enteric nervous system fails to develop, and animals display an aganglionic megacolon that resembles Hirschsprung's disease in humans[117]. In melanoma cells, ET3, similarly to ET1, promotes invasive behaviour and survival through ETBR[39,44,118], and in rhabdomyosarcoma cells, ET3 can act as a paracrine factor that promotes the migration of endothelial cells[119]. Evidence suggests that the three endothelin peptides have somewhat distinct activities and outcomes depending on cellular context, although it is thought that the mechanistic details of their actions are similar. In fact, there is currently no clarity regarding specificity in endothelin signalling with respect to each endothelin isoform. Recent evidence has suggested that distinct cell populations within a tumour can express distinct isoforms. For example, in glioblastoma, the cancer stem cell population displayed *EDN3* expression and activity, whereas the more differentiated cancer cells expressed *EDN1* (REF. 49). In breast and cervical cancer tissues, expression of *EDN3* is substantially reduced[51,52], suggesting that ET1 may even have an opposing tumour suppressor function in these tumours. If all three endothelin isoforms function in a mechanistically similar manner, how can these different activities be explained? Although much work needs to be done to answer these questions, it is intriguing to speculate that the different activities among the endothelin peptides are primarily mediated by epigenetic events[120]. Mechanistic studies revealed hypermethylation and downregulation of *EDN2* and *EDN3* in human primary colon cancers and in a panel of colon cancer cell lines[121]. In this regard, epigenetic inactivation of *EDN3*, which occurs frequently in human colon[121], breast and cervical cancers[51,52], may be a necessary step that leads to cancer. On the basis of these observations, it is possible to hypothesize that *EDN2* and *EDN3* might be silenced early in cancer development to circumvent competition with ET1 for its receptors. These findings could open a new avenue for potential roles of ET2 and ET3 in cancer, providing further support for the use of non-selective dual ETAR and ETBR antagonists, which are also capable of blocking the activity of ET2 and ET3 signalling.

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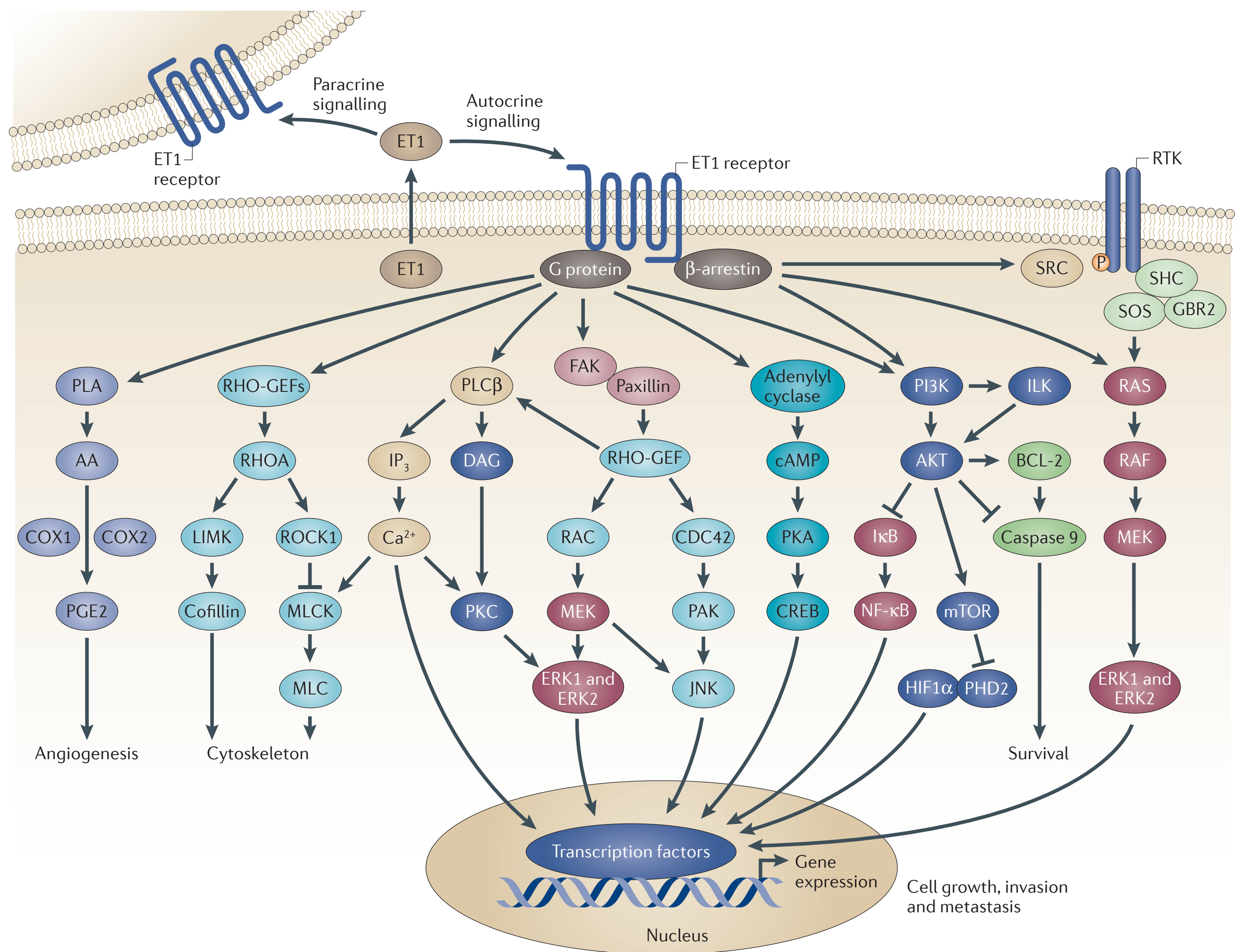

Figure 1 | **The ET1 signalling network.** The endothelin 1 (ET1) receptor (usually endothelin A receptor (ETAR) but sometimes ETBR) couples to one or more families of G proteins, as well as to scaffold proteins, such as β-arrestins. This activates diverse signal-transduction pathways, including phospholipase Cβ (PLCβ), which cleaves phosphatidylinositol-4,5-bisphosphate ($PtdIns(4,5)P_2$) into diacylglycerol (DAG) and inositol triphosphate (IP_3), leading to calcium mobilization and protein kinase C (PKC) activation, and activation of downstream members of the MAPK family, including ERK signalling. ET1 receptor signalling also controls the crosstalk between ET1 receptors and receptor tyrosine kinases (RTKs) through the recruitment and activation of SRC, resulting in downstream pathway activation. Moreover, ET1 receptor stimulation activates phospholipase A (PLA) and arachidonic acid (AA) and downstream cyclooxygenase 1 (COX1) and COX2, leading to prostaglandin E2 (PGE2) production. ET1 receptor signalling also activates focal adhesion kinase (FAK)–paxillin, resulting in the activation of RHO-guanine nucleotide exchange factor (RHO-GEF) and PI3K, leading to AKT, integrin-linked kinase (ILK) and mTOR activation and inhibition of prolyl hydroxylase 2 (PHD2), which stabilizes hypoxia-inducible factor 1α (HIF1α) activity. ET1 activates nuclear factor-κB (NF-κB) signalling via inhibition of NF-κB inhibitor (IκB), resulting in the dissociation and subsequent nuclear localization of active NF-κB. At the same time, ET1 receptor activation stimulates RHO-GEF and a small GTPase protein, RAS homology A (RHOA), initiating RHO-dependent signalling events through RHO-associated coiled-coil containing protein kinase 1 (ROCK1), causing cytoskeletal remodelling. cAMP, cyclic AMP; CREB, cAMP-responsive element-binding; GRB2, growth factor receptor-bound 2; JNK, JUN N-terminal kinase; LIMK, LIM domain kinase; MLC, myosin light chain; MLCK, MLC kinase; PAK, p21-activated kinase; PKA, protein kinase A; SHC, SRC homology 2 domain-containing.

Lymphangiogenesis
The formation of lymphatic vessels from pre-existing lymphatic vessels, in a way similar to blood vessel development or angiogenesis.

inducing apoptosis. When combined with conventional chemotherapy, ETAR-selective antagonists (such as atrasentan and zibotentan) or dual ETAR and ETBR antagonists (such as macitentan) can more effectively induce apoptosis *in vitro* and *in vivo*, as observed in ovarian, prostate, colon and cervical cancer cells[22–31].

ET1 and the tumour microenvironment

In addition to modulating tumorigenesis, ET1 seems to have a role in tumour progression, including migration, invasion, epithelial-to-mesenchymal transition (EMT), metastatic growth, angiogenesis and lymphangiogenesis.

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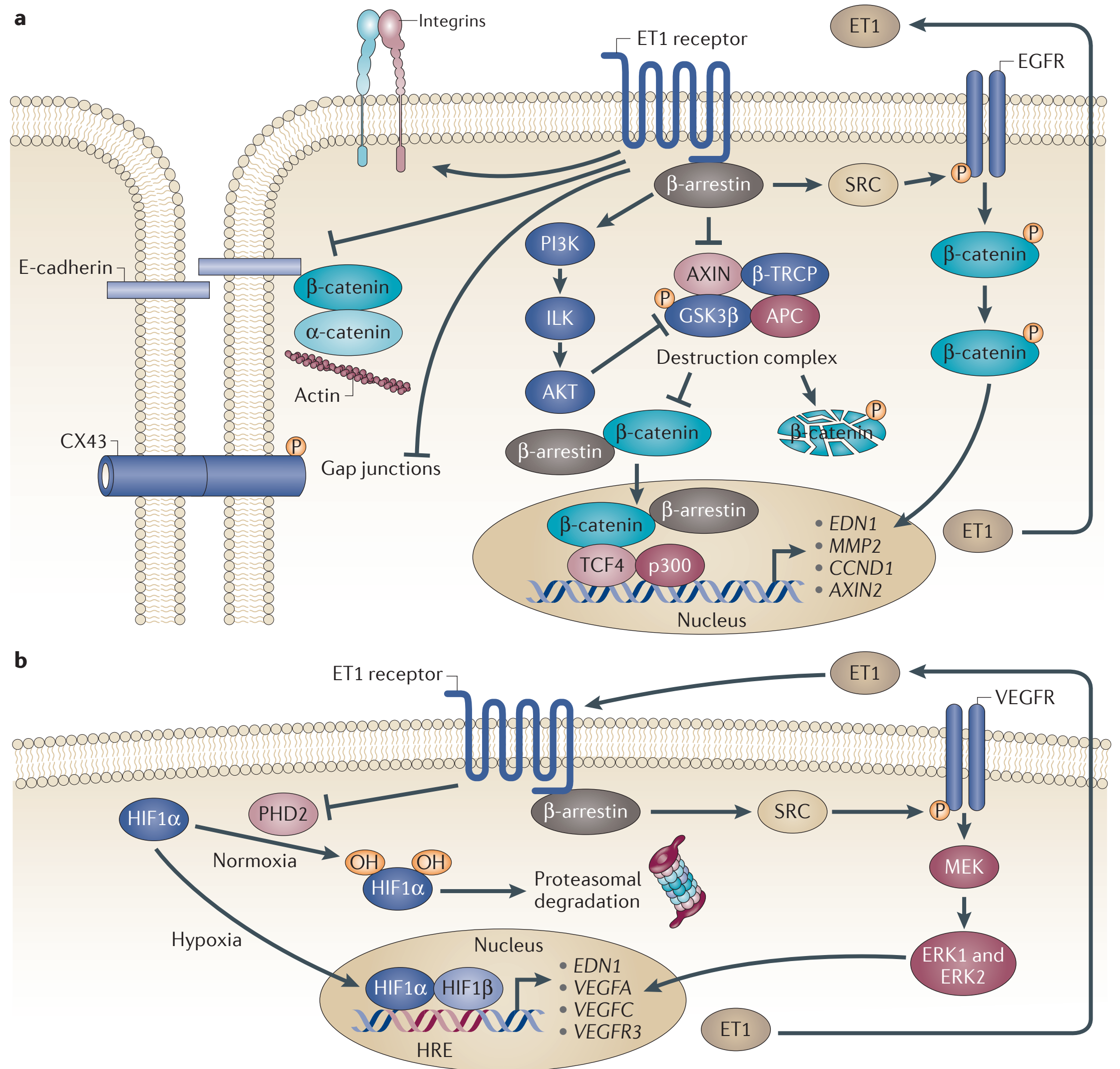

Figure 2 | **Crosstalk between ET1 signalling and the β-catenin and HIF1α signalling pathways. a** | Cooperation between endothelin 1 (ET1) and β-catenin signalling is shown. The activation of ET1 receptors (usually endothelin A receptor (ETAR) but sometimes ETBR) controls host–tumour interactions by downregulating the membrane adherens junction components, such as epithelial cadherin (E-cadherin) and β-catenin, by increasing integrin subunit expression and by regulating gap junction intercellular communication through connexin 43 (CX43) phosphorylation (P), thus enhancing cell adhesion, migration and invasiveness. In ovarian cancer cells, ET1 controls β-catenin through several coordinated mechanisms. Binding of ET1 to ETAR leads to the recruitment of β-arrestin to the activated receptor. As a signal transducer, β-arrestin mediates the PI3K–integrin linked kinase (ILK)–AKT signalling route, which causes the inhibition of the β-catenin destruction complex through the phosphorylation and inactivation of glycogen synthase kinase 3β (GSK3β). This results in the accumulation of a non-serine/threonine phosphorylated, active β-catenin. In a parallel and coordinated manner, the ETAR–β-arrestin complex binds axis inhibition 1 (AXIN1), thereby promoting the inactivation of GSK3β. Concomitantly, ETAR–β-arrestin associates with SRC and triggers the transactivation of the epidermal growth factor receptor (EGFR) and subsequent tyrosine phosphorylation of β-catenin, contributing to the activation and nuclear translocation of β-catenin. In addition, in an ET1-dependent manner, β-arrestin 1 shuttles with β-catenin into the nucleus and interacts with p300 histone acetyltransferase to enhance the transactivation activity of β-catenin and T cell-specific transcription factor 4 (TCF4), thus promoting the transcription of genes, such as endothelin 1 (*EDN1*; which encodes ET1), matrix metalloproteinase 2 (*MMP2*), cyclin D1 (*CCND1*) and *AXIN2*. Given that ET1 is a downstream target of β-catenin–TCF4, the cooperation between β-catenin and ET1 results in the autoregulatory β-catenin-mediated transcription of *EDN1*. **b** | Cooperation between ET1 and hypoxia signalling is shown. The ET1 receptors regulate hypoxia-inducible factor 1α (HIF1α) at the levels of transcription and protein stability. In particular, ET1 increases HIF1α stability by inhibiting prolyl hydroxylase 2 (PHD2) activity, which controls O_2-dependent degradation of HIF1α through hydroxylation (OH). HIF1α accumulation leads to nuclear entry and binding to hypoxia-response elements (HREs) and the transcription of genes, such as vascular endothelial growth factor A (*VEGFA*) and *VEGFC* and their receptor, VEGF receptor 3 (*VEGFR3*), as well as *EDN1*. The increased ET1 expression following HIFα activation establishes a positive interplay with hypoxia. ET1 receptor activation triggers the recruitment of β-arrestin 1 to the receptor that controls the crosstalk between ET1 receptor and VEGFR3 through the recruitment and activation of SRC, leading to ERK signalling. APC, adenomatous polyposis coli; β-TRCP, β-transducin repeat-containing protein.

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Triple-negative breast cancer
(TNBC). Breast cancer that lacks the expression of oestrogen receptor, progesterone receptor and ERBB2.

Proenzymes
Also known as zymogens. The inactive or nearly inactive precursors of enzymes, which are converted into active enzymes by proteolysis.

Mesothelial
Pertaining to the layer of flat cells of mesodermal origin that lines the embryonic body cavity and that gives rise to the squamous cells of the peritoneum, pericardium and pleura.

Migration and invasion. During progression, tumour cells are stimulated to release proteases, to lose polarity and cell–cell junctions, to acquire a mesenchymal phenotype, to invade extracellular matrix (ECM) and to migrate to distant sites. These changes are characterized by the disassembling of gap junction intercellular communications (GJICs), tight junctions and adherens junctions, the reorganization of cell substrate adhesion complexes, and a substantial remodelling of the cytoskeleton[32]. Recently, the role of ET1 in tumour invasion has been investigated in triple-negative breast cancer (TNBC)[33]. The lactoferrin-mediated increase in the invasiveness of breast cancer cells is mediated through increased expression and secretion of ET1, and these changes might account for the generally noted aggressive behaviour of TNBC. Moreover, the addition of BQ-123 to breast cancer cell cultures abrogated the ability of lactoferrin and ET1 to promote cell migration and invasion, suggesting that targeting the lactoferrin–ET1 axis in TNBC could be a promising new therapeutic approach[33].

Some mechanistic insights into ET1-driven ECM degradation are emerging. ET1 regulates two families of metastasis-related proteinases — the MMPs and the urokinase-type plasminogen activator system — at several levels, including gene transcription and the secretion and activation of the resultant proenzymes. For example, in tumour cells, ET1 activates soluble MMPs, such as MMP2, MMP9, MMP3, MMP7 and MMP13. ET1 also inceases the activation of membrane type-1-MMP (MT1-MMP) and decreases the secretion of tissue inhibitor of MMP1 (TIMP1) and TIMP2, increasing the MMP/TIMP ratio and causing the rapid degradation of the ECM[34,35].

ET1 signalling also contributes to increased tumour cell motility and actin stress fibre disorganization by increasing the activation of p125 focal adhesion kinase (FAK), paxillin and RHO, which have been shown to transduce signals that are involved in tumour cell invasion[14,36–38]. Increased cell motility depends on the regulated expression of different integrins that mediate the attachment to an underlying ECM, as well as delivering intracellular messages to modulate cellular functions and behaviour through crosstalk with growth factors, including ET1. This crosstalk involves the activity of integrin-linked kinase (ILK), an intracellular serine/threonine kinase. ILK, through direct binding to the cytoplasmic domains of β1 integrin subunits, connects integrins to the actin cytoskeleton, coordinating cell spreading and actin organization that is required for cell motility[38]. The capacity of ET1 to modulate the changes in expression of α2β1 and α3β1, as well as α2β1 and αvβ3 integrins, has been shown in ovarian cancer and melanoma cells[38,39], respectively. In ovarian cancer cells, the interaction of β1 integrin with type I collagen, the unique protein of the mesothelial ECM in the peritoneal cavity, increases ILK activity, and ET1 may mimic this signal and cooperate with β1 integrin to activate ILK and expand the cellular communication signalling network that leads to tumour cell motility and invasion[38]. Melanoma cells engage and cluster β1 integrins at the leading edge, which results in polarized ECM degradation and collective migration and invasion. In these cells, ET1 is also capable of upregulating β1 integrin, MT1-MMP and MMP2 expression, which is accompanied by increased rates of adhesion to ECM molecules and by increased migratory and invasive capacity. Antibodies directed to α2β1 and αvβ3 integrins strongly reduced adhesion to type I collagen, as well as cell migration induced by ET1 (REF. 39).

Following malignant transformation, stepwise changes in GJICs enable tumour cells to escape microenvironmental control from the normal surrounding tissue, thus promoting local invasiveness and metastatic spread. GJIC formation requires the alignment of two hexameric connexin hemi-channels (one in each cell membrane of the interacting cells). Defects in intercellular communication, including reduced or inappropriate expression of connexin, predominantly connexin 43 (CX43; also known as GJA1), have emerged as key factors in tumour progression. In ovarian carcinoma and in melanoma cells, ET1, via ETAR and ETBR, respectively, induces phosphorylation of CX43 through the SRC pathway, leading to cellular uncoupling and indicating that ET1 promotes this reduced coupling at the level of post-translational connexin modification[39,40]. The capacity of ET1 to disrupt intercellular communications, combined with the effects on ECM attachment, underlines the relevance of ET1 receptors in regulating cell–cell or cell–matrix interactions during malignant progression.

EMT, stemness and therapy resistance. Multiple extracellular signals can initiate an EMT-related gene expression programme through substantial crosstalk among the downstream intracellular signalling pathways and transcription factors that choreograph this complex network, including positive-feedback loops that can lead to stable reprogramming of epithelial cells to mesenchymal states. Activation of the ET1 pathway may induce EMT through multiple distinct signalling mechanisms, including the upregulation of the mesenchymal-associated transcription factor SNAIL (which is encoded by *SNAI1*)[41,42]. ET1 can increase *SNAI1* mRNA levels, SNAIL protein stability and transcriptional activity; this is accompanied by the downregulation of epithelial cadherin (E-cadherin) mRNA, which is an epithelial marker and a direct transcriptional target of SNAIL[41,43]. In addition to SNAIL, ET1 stabilizes the β-catenin protein in a coordinate manner (as discussed above) to cooperatively engage transcriptional programmes of genes, such as *EDN1*, *MMP2* and *AXIN2,* leading to EMT[16,21] (FIG. 2a).

Hypoxia is one of the physiological factors that can induce EMT in tumours through distinct mechanisms, including the upregulation of HIF1α[32]. The ET1 axis has been shown to be regulated by, and to induce the activation of, HIF1α (discussed further below in the context of angiogenesis)[44,45], suggesting that this ET1 autocrine loop contributes to the complex cooperation between the intracellular signalling pathways and extracellular signals to trigger EMT.

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Interactions between tumour cells and the microenvironmental niche can trigger not only EMT programmes but also the maintenance of stem-cell-like characteristics[46]. ET1 expression has been associated with features expressed by a highly tumorigenic subpopulation of cancer stem cells (CSCs; also known as tumour-initiating cells)[47–50]. High levels of ET1 are expressed in putative CSCs that are isolated as a subpopulation of colon cancer cells expressing the CD133 stemness marker[49]. A gene expression analysis identified a different endothelin, *EDN3*, as one of the most overexpressed transcripts in CD133$^+$ glioblastoma stem cells (GSCs), when compared with autologous differentiated glioblastoma cells[48]. Interestingly, in GSCs, upregulation of *EDN3* is coupled with downregulation of *EDN1*, whereas in human glioblastoma tumours there is increased expression of *EDN1* but not of *EDN3* (REFS 48,49). This suggests that, in comparison to ET1, ET3 may have a tumour-suppressive role by maintaining the quiescence of GSCs and so limiting their tumorigenic potential, as has been similarly observed in breast and cervical cancers[51,52] (BOX 1). Autocrine signalling involving ET3 and ETBR is a mechanism for the survival and maintenance of GSC pools, and ET3–ETBR blockade with BQ-788 weakens the tumorigenic potential of GSCs[49]. These findings support the view that the prevention of GSC-mediated tumour recurrence may require the targeting of active stem cell pathways in GSCs, such as the ET3–ETBR pathway.

Emerging evidence suggests molecular and phenotypic associations between chemoresistance and the acquisition of CSC and EMT phenotypes in cancer cells[32]. Therefore, targeting CSCs or cells that have an EMT phenotype by antagonizing specific receptors may increase the sensitivity of tumour cells to conventional chemotherapeutics[32,46]. Interestingly, chemoresistant ovarian cancer cells showed phenotypic changes that were consistent with EMT and stemness, thus reinforcing the close relationship of both processes with therapy resistance[47,53,54]. In these cells, increased ETAR expression levels were associated with higher expression of the mesenchymal markers SNAIL, SLUG, TWIST, vimentin and neural cadherin compared with parental cells, and this was accompanied by a concomitant decrease in E-cadherin expression[55]. The blockade of ETAR reversed EMT, inhibited invasiveness and restored chemotherapy sensitivity. In mouse xenografts of human ovarian cancer cells that were sensitive or refractory to chemotherapy, zibotentan inhibited tumour growth and sensitized cells to chemotherapy, suggesting that a combination therapy may be effective[55]. Analysis of human ovarian cancer tissues with a range of sensitivities to chemotherapy showed that the resistant cells displayed ETAR overexpression and EMT marker expression[55]. Of note, ETAR is preferentially expressed in CD133$^+$ ovarian cancer cell lines and primary human tumour cells, hence ETAR may play a part in the chemoresistance of CSCs[47]. Taken together, these observations indicate that ETAR expression may be a predictor of chemoresistance, and that targeting ETAR could increase the sensitivity of tumours to therapeutic agents[47,55,56].

Metastasis. Several studies in model systems have described a broad range of potential ET1 effects on metastatic colonization. In a mouse orthotopic model of metastatic human ovarian cancer, silencing of β-arrestin 1 or treatment with zibotentan inhibited metastasis by preventing nuclear β-arrestin 1–β-catenin association and by inhibiting the transcription of EMT- and metastasis-related genes[21]. This indicates that the metastatic phenotype is linked to the ETAR–β-arrestin-mediated β-catenin pathway. In a similar model, the administration of the dual ET1 receptor antagonist macitentan in combination with paclitaxel prevented the progression of ovarian cancer by inhibiting survival pathways both in tumour cells, which express ETAR, and in tumour-associated endothelial cells, which express ETBR[30,31].

Overexpression of ETBR increased the incidence of brain metastases in a preclinical model of spontaneous metastasis of melanomas to the central nervous system (CNS)[57]. Activation of ETBR by ET1 enhanced tumour cell proliferation and intracranial melanoma growth that was inhibited by the ETBR antagonist A192621. The identification of an influential role of ETBR in spontaneous melanoma–CNS metastasis may provide both a target for therapeutic intervention and a potential prognostic marker to identify patients with melanoma who have an increased risk of brain metastasis[57].

Recent studies demonstrated that ET1 in the bladder cancer microenvironment primes cancer cells for pulmonary metastasis[58,59]. Gene expression analyses identified *EDN1* as a crucial gene suppressed by RHO GDP-dissociation inhibitor 2 (RHO-GDI2), a metastasis suppressor. In bladder cancer cells expressing low levels of RHO-GDI2 and secreting ET1, metastatic lung colonization was inhibited by atrasentan treatment[58]. Levels of ET1 are higher in patients with muscle-invasive bladder cancers, which are prone to metastasize and which correlate with reduced patient survival, thus indicating ET1 as a biomarker for lung metastasis[59]. Indeed, tumour-derived ET1 functioning through ETAR enhances the migration and invasion of both tumour cells and macrophages and induces the expression of inflammatory cytokines and proteases[59]. Zibotentan treatment significantly reduced the subcutaneous growth of bladder cancer cells, but only when the drug was administered before cell inoculation. Of note, zibotentan treatment dramatically reduced the development of lung metastases, and this was associated with a significant decrease in the numbers of tumour-associated macrophages (TAMs), indicating that ET1–ETAR triggers macrophage infiltration and inflammatory cytokine production in the lung before the development of metastases[59]. These results, which demonstrate that zibotentan reduces the early inflammatory response and the subsequent development of metastasis before the cancer cells lodge at the metastatic colonization site, suggest that ETAR inhibitors might be more effective as adjuvant therapeutic agents before metastases become clinically apparent[59].

Recent studies have uncovered a prominent role for ET1 in the formation of osteoblastic lesions, which are frequently observed in patients with metastatic

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Osteoblasts
Cells responsible for bone formation. They express bone sialoprotein and osteocalcin and produce osteoid, which is mainly composed of type I collagen.

Osteoclast
A cell that breaks down mineralized bone and that is responsible for bone resorption.

prostate cancer and, to a lesser extent, in those with metastatic breast cancer. In the bone microenvironment, tumour-derived ET1 induces new bone formation by stimulating mitogenesis in osteoblasts, which express both ETAR and ETBR, and by decreasing osteoclast activity and motility[60–63]. Although the precise mechanisms of action remain to be elucidated, gene expression analysis in mouse primary osteoblasts revealed that treatment with ET1 upregulates multiple osteoblast-stimulatory factors — such as *Il6*, *Wnt5a*, connective tissue growth factor and receptor activator of NF-κB ligand — and downregulates the WNT signalling pathway inhibitor Dickkopf-related 1 (REF. 62). Bone metastasis in experimental models is inhibited by the ETAR antagonists atrasentan and zibotentan, as well as by the dual ETAR and ETBR antagonist bosentan[63]. Evidence is also accumulating to suggest that ET1 contributes to the pain that is associated with metastatic disease. This occurs through ET1 causing the excitation of nociceptors through ETAR but this also concurrently produces analgesia through ETBR-mediated β-endorphin release and opioid receptor activation[64,65]. Indeed, selective antagonism of ETAR has been shown to ameliorate pain[66]. Overall, therefore, ETAR blockade could represent a therapeutic option to reduce bone metastasis and associated pain.

Angiogenesis and lymphangiogenesis. Angiogenesis and lymphangiogenesis are both controlled by cellular and environmental regulators, including local hypoxia. ET1 is mitogenic for blood and lymphatic endothelial cells, vascular smooth muscle cells, fibroblasts and pericytes[1], and ETBR controls the proliferation of blood and lymphatic endothelial cells[67,68]. During the formation of new blood and lymphatic vessels, endothelial cells are stimulated to release proteases, such as MMP2, which allow the endothelial cells to migrate, proliferate and invade surrounding tissues to form capillaries. ET1, through ETBR, induces these various stages of neovascularization and displays a potent additive effect with VEGF family members in the angiogenic and lymphangiogenic processes[67,68]. Although ET1 can modulate angiogenesis and lymphangiogenesis independently of VEGF, it can also act through the induction of VEGF[44,67–69]. In different tumour types, increased expression of ET1 and its cognate receptors is significantly associated with the expression of VEGF and its receptors — VEGFR1 (also known as FLT1) and VEGFR2 (also known as KDR) — as well as with microvessel density[70]. This indicates that ET1 and VEGF might have complementary and coordinated roles during neovascularization.

Expression of ET1, ETAR and ETBR has been correlated with increased lymphatic dissemination[71,72], and lymphatic vessels in human lymph nodes express ETBR[67]. Furthermore, ET1 promotes the outgrowth of functional newly formed lymphatic vessels, which is blocked by BQ-788 (REF. 67). These results, together with a gene expression profile identifying *EDN1* as one of the significantly upregulated genes in lymphatic endothelial cells isolated from metastatic lymph nodes[73,74], indicate that ET1–ETBR may directly modulate lymphangiogenesis.

Various studies are uncovering the molecular basis of the involvement of ET1 in cancer cells and endothelial cells in response to a hypoxic microenvironment, particularly involving a reciprocal relationship between ET1 and HIF1α, which is a crucial hypoxia-responsive transcriptional factor subunit. Under normoxic conditions, a HIF prolyl hydroxylase domain (PHD) protein catalyses a site-specific hydroxylation of HIF1α to trigger its proteasomal degradation. During hypoxia, the absence of this hydroxylation stabilizes HIF1α, enabling it to form a dimeric HIF transcription factor complex that binds to hypoxia-response element (HRE)-binding sites to induce the transcription of pro-angiogenic genes, including those of VEGF family members. Degradation of HIF1α was found to be reduced in ET1-treated tumour cells under both hypoxic and normoxic conditions[45,75]. Mechanistically, ET1 seems to stabilize HIF1α by downregulating PHD2 (REF. 75). Following this stabilization, the HIF1 transcription complex is formed at HREs, which induces the expression of *VEGFA* and the selective lymphangiogenic factors *VEGFC* and *VEGFD*, as well as the expression of their receptor, *VEGFR3*, especially during hypoxia[44,67] (FIG. 2b). Thus, ET1 potentiates the response to hypoxia by amplifying HIF1α stability and VEGF production. Interestingly, HIF1α also activates the transcription of *EDN1* in different cell types. Therefore, an autocrine ET1 circuit is controlled by the tumour microenvironment, and ET1 itself modifies that environment through HIF1α stabilization[45,76,77] (FIG. 2b).

Additionally, in hypoxic conditions, ET1 increases the promoter activity of cyclooxygenase 1 (*COX1*) and *COX2*, and the production of prostaglandin E2 (PGE2). COX1 and COX2 function as downstream mediators of ET-induced angiogenic and invasive properties[78]. Silencing of *HIF1A* by small interfering RNA abrogates ET1-induced *COX2* transcriptional activity, PGE2 and VEGF production and MMP activation, thus implicating both *HIF1A* and *COX* genes as downstream targets of ET1 signalling[78]. More recently, studies evaluating the functional contributions of mesenchymal stem cells to tumour angiogenesis demonstrated that IL-6 that is secreted by tumour mesenchymal stem cells may enhance the secretion of ET1 by cancer cells to increase angiogenesis[79].

The generation of capillary-like vessels by genetically deregulated, aggressive tumour cells has been termed vasculogenic mimicry to emphasize their *de novo* generation, without the participation of endothelial cells and independent of angiogenesis. Gene expression profiling of melanoma cell lines has identified the gene encoding ETBR as one of the genes associated with a more aggressive phenotype, which is characterized by the capacity to organize vasculogenic mimicry[80]. Thus, ET1, through ETBR, promotes the formation of a tubular network of melanoma cells, and this pathway has also been shown to cooperate with VEGFC to trigger vasculogenic mimicry[17].

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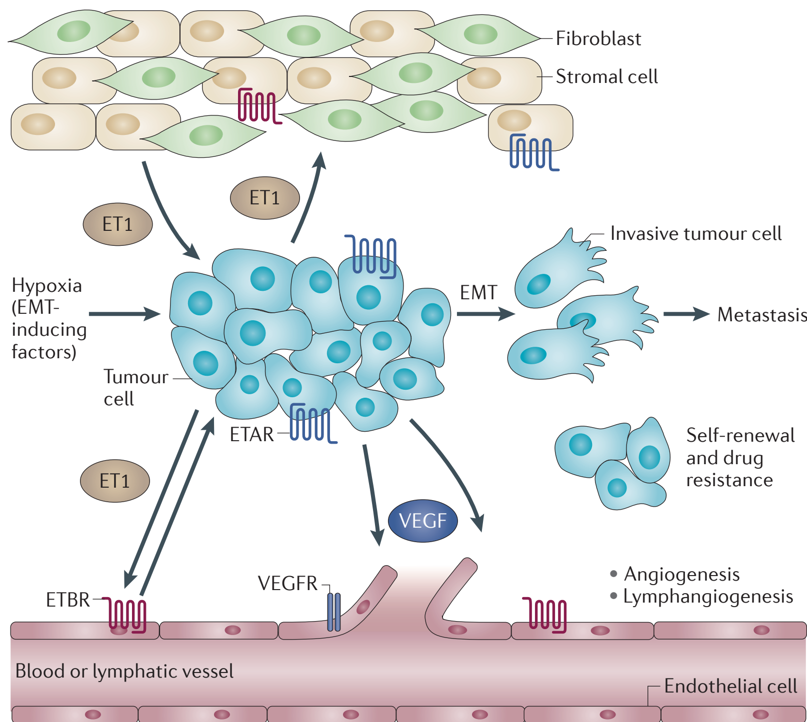

Figure 3 | **The ET1-regulated tumour–microenvironment interactions in tumour maintenance and progression.** Tumour cells express endothelin 1 (ET1) receptors (usually endothelin A receptor (ETAR) but sometimes ETBR) and secrete ET1, thus activating an autocrine loop and paracrine tumour–stroma interactions. ET1 might modulate tumour stroma remodelling by acting on both ETAR and ETBR expressed on cancer-associated fibroblasts. In turn, the tumour receives cues from the stroma, including epithelial-to-mesenchymal transition (EMT)-inducing factors and hypoxic stimuli, in response to which the tumour cells acquire invasive or stem cell-like properties, including self-renewal and drug-resistance properties. Some of these cells may acquire both properties (possibly owing to activated ET1 signalling) and be able to metastasize and establish secondary tumours. Blood and lymphatic endothelial cells increase angiogenesis and lymphangiogenesis in response to ET1–ETBR activation. In parallel, the tumour secretes vascular endothelial growth factor (VEGF) in an ET1-dependent manner, inducing sprouting and branching of new vessels from existing vessels. VEGFR, VEGF receptor.

In view of the cooperation of ET1 with members of the VEGF family in the vasculogenic process, interfering with ETBR, using a dual ETAR and ETBR antagonist, might offer an attractive ‘two-hit’ therapeutic strategy. This strategy would act on aggressive tumour cells expressing ETBR by impairing vasculogenic mimicry and other tumorigenic effects mediated by this receptor, as well as on blood and lymphatic endothelial cells by affecting angiogenesis and lymphangiogenesis, which are important routes for the metastatic spread of cancer cells (FIG. 3).

Inflammation, macrophages and fibroblasts. ET1 modulates trafficking, differentiation and activation of tumour-infiltrating immune cells, such as TAMs. These cells, which express ET1 receptors and produce ET1, migrate towards ET1 because of their expression of ETBR. In bladder cancer, ET1 acting on ETAR triggers macrophage influx, and production of pro-inflammatory mediators, such as IL-6, chemokine (C-C motif) ligand 2 (CCL2), COX2 and MMPs, which breach vascular integrity and enable the dissemination of cancer cells to the lungs[59]. Interestingly, metastasis is suppressed by macrophage depletion and by zibotentan[59]. Moreover, ET1 that is secreted by tumour cells is a paracrine regulator of the tumour stroma. ET1 acts on both ETAR and ETBR, which are expressed on fibroblasts that have been isolated from normal tissues adjacent to human colon[81,82], ovarian[10] and breast[83,84] cancers. Treating the colon-cancer-adjacent fibroblasts with ET1 *in vitro* promoted fibroblast growth, migration, contraction and the production of ECM-modifying proteins[81]. This suggests that ET1 may act on cancer-associated fibroblasts to promote the formation of a supportive tumour stroma. Therefore, the ET1-driven crosstalk between tumour cells, macrophages and fibroblasts induces a specialized niche that sustains tumour maintenance and progression (FIGS 3,4).

Lymphocytes and dendritic cells. The most efficient antigen-presenting cells are immunologically competent dendritic cells, which produce ET1 and which express functional ETAR and ETBR, which are both highly expressed during dendritic cell maturation[85]. Interestingly, ETAR and ETBR seem to have opposite effects on dendritic cell function and survival. ETAR blockade by atrasentan significantly reduces the expression of the mature dendritic cell marker CD83, decreases the production of IL-12, downregulates the ability of dendritic cells to stimulate T cells and promotes dendritic cell apoptosis. By contrast, ETBR blockade by A192621 results in increased expression of CD83 and improved dendritic cell survival. Therefore, autocrine or paracrine circuits involving ET1, ETAR and ETBR seem to regulate the maturation and function of dendritic cells[85].

The role of ETBR in cancer immunotherapy has been thoroughly investigated in ovarian cancer cells[86–88], in which ETBR-mediated signalling has been shown to inhibit the homing of T cells to tumours, and this effect was suppressed by ETBR antagonists. Indeed, ETBR overexpression is associated with a loss of tumour-infiltrating lymphocytes. Moreover, ET1 abrogates adhesion of T cells to the endothelium, and this occurs through ETBR-mediated suppression of endothelial intercellular adhesion molecule 1 (ICAM1) expression[86–88]. Altogether, these observations strongly underline that dual ETAR and ETBR antagonists might offer the advantage of simultaneously targeting the tumour cells (through ETAR) and enhancing antitumour immune mechanisms (through inhibiting vascular ETBR) by augmenting T cell infiltration, as well as by increasing the homing of T cells to tumours (FIG. 4).

Therapeutic opportunities and clinical trials

Various findings have provided a rationale for the therapeutic targeting of ET1 signalling: the activation of autocrine or paracrine ET1 signalling in many tumorigenic processes, including tumour initiation, metastatic colonization and resistance to therapeutic

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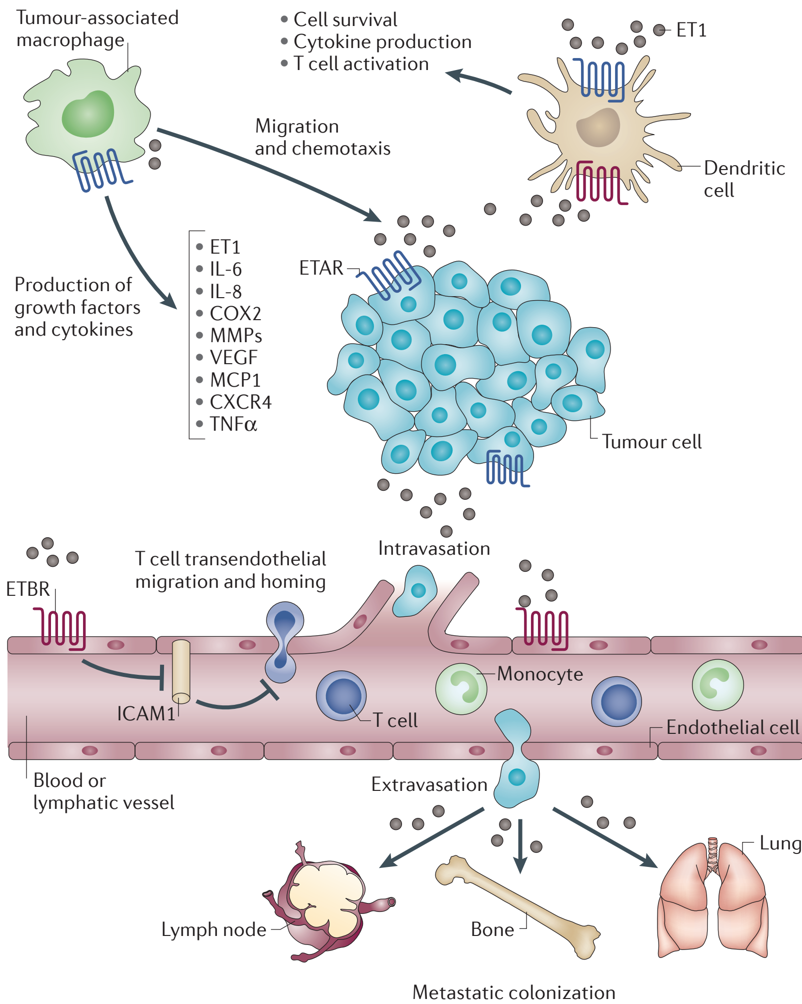

Figure 4 | **The ET1 axis in immunomodulation.** A local endothelin 1 (ET1) axis can augment the response of immune cells in the tumour microenvironment. In particular, ET1-driven autocrine or paracrine loops modulate dendritic cell function, such as increases in cell survival, cytokine production and T cell activation. Moreover, ET1 acting through ET1 receptors expressed on tumour-associated macrophages activates pro-inflammatory transcription factors and stimulates the production of inflammatory cytokines, triggering macrophage infiltration and further secretion of inflammatory cytokines, which is a necessary step for intravasation, extravasation and metastatic colonization. In turn, tumour cells, in response to chemokine stimulation, migrate towards the chemoattractant gradient until they reach the site for secondary colonization. ET1 receptor activation also causes increased release of vascular endothelial growth factor (VEGF), which is important for tumour cell extravasation and angiogenesis. Concomitantly, during tumour progression, ET1, through endothelin B receptor (ETBR) overexpressed in tumour endothelium, controls the endothelial barrier to T cell homing to tumours, by inducing the suppression of intercellular adhesion molecule 1 (ICAM1) expression on endothelial cells, which mediates T cell transendothelial migration and homing to tumours. COX2, cyclooxygenase 2; CXCR4, C-X-C chemokine receptor type 4; IL, interleukin; MCP1, monocyte chemotactic protein 1; MMPs, matrix metalloproteinases; TNFα, tumour necrosis factor-α.

agents; the aberrant activation of ET1 signalling in a wide range of cancers, including ovarian, prostate, colon, breast, bladder and lung cancers[25,55,59,63,89–97]; and a correlation between ET1 receptor expression and pathological outcomes, such as patient survival and metastasis in various human malignancies (TABLE 1).

As ETAR is the main endothelin receptor that is overexpressed in cancers (TABLE 1), therapeutic approaches have generally focused on the selective ETAR antagonists and the dual-specific ETAR and ETBR antagonists discussed above. Following encouraging preclinical activity, the specific ETAR antagonist zibotentan, the selective ETAR antagonists atrasentan and YM-598, and the dual ETAR and ETBR antagonists bosentan and macitentan have been evaluated in clinical oncology settings[98], and key results are described below (TABLE 2). Other less well-developed ET1-targeting approaches include the selective ETBR agonist IRL-1620, which increases tumour perfusion to potentiate the therapeutic efficacy of anticancer agents[99] and of radiation therapy[100]; the selective inhibition of ET1 synthesis by ECE1 inhibitors or by natural products, such as green tea[101] and red wine biologically active polyphenols[102]; and the modulation of ET1 degradation by upregulating NEP activity[103,104].

Atrasentan and zibotentan have demonstrated potential anticancer activity in compelling preclinical studies in prostate cancer models[98,105]. Following promising Phase II trials in patients with advanced metastatic prostate cancer, both compounds have been evaluated as single agents in Phase III trials carried out in patients with non-metastatic prostate cancer or advanced metastatic prostate cancer and in combination with standard chemotherapy (docetaxel) in patients with metastatic prostate cancer. Unfortunately, all trials have proved to be unsuccessful in patients with established disease. In particular, the study evaluating zibotentan monotherapy in patients with non-metastatic prostate cancer was stopped prematurely after an early efficacy review indicated that it was unlikely to meet its primary objectives of improved overall survival and progression-free survival (PFS)[106]. During recruitment for this trial, the screening of patients for non-metastatic disease status failed in an unexpectedly high number of patients, who were then ineligible for the study[106,107]. Further investigation revealed that the leading cause of screening failure was the high-frequency detection of previously unidentified asymptomatic metastasis by medical imaging methods, thus highlighting the importance of periodic staging assessments for the design of optimal treatment modalities for metastatic and non-metastatic disease[107]. It has been speculated that the negative trial results obtained underpin the observation that pharmacological inhibition of ETAR does not affect established high-volume disease because these advanced tumours are no longer dependent on macrophages or their inflammatory response for maintenance and growth[59]. Overall, this suggests that ETAR antagonists should be evaluated in the adjuvant setting in patients with non-metastatic disease, with the aim of hampering metastatic colonization by regulating key factors in the tumour microenvironment[59].

Extensive preclinical evidence of antitumour activity of zibotentan in ovarian cancer models has propelled the clinical investigation of this agent. A Phase II trial assessing zibotentan plus carboplatin and paclitaxel in 120 patients with advanced ovarian

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Table 1 | **The expression of endothelin receptors in human malignancies**

Tumour type	Endothelin receptor expressed	Phenotypes associated with endothelin receptor expression
Bladder	ETAR[59] and ETBR[122]	Reduced patient survival[59]
Glioblastoma	ETAR and ETBR[123,124]	NR
Breast	ETAR[71,125]	Reduced patient survival and increased bone metastases[71,125]
Cervical	ETAR[52]	NR
Colorectal	ETAR[126,127]	Increased tumour grade and reduced patient survival[127]
Hepatocellular	Loss of ETBR[128]	NR
Gastric	ETAR[129] and loss of ETBR[130]	NR
Head and neck	ETAR[96]	Reduced patient survival[131]
Lung	ETAR (NSCLC)[132] and ETBR (SCLC)[133]	Reduced patient survival (NSCLC)[132]
Melanoma	ETBR[80]	Aggressive phenotypes and metastasis to the lymph nodes[80,134,135]
Ovarian	ETAR[136]	Increased tumour grade, chemoresistance and metastasis[21,41,54,136]
Prostate	ETAR[2]	Increased tumour grade and bone metastases[2,137,138]
Renal	ETAR[139] and ETBR[140,141]	ETAR is associated with tumour grading[139] and ETBR is associated with patient survival[140–142]
Vulvar	ETBR[143]	Reduced patient survival[144]

ETAR, endothelin A receptor; ETBR, endothelin B receptor; NR, not reported; NSCLC, non-small-cell lung cancer; SCLC, small-cell lung cancer.

cancer reported no improvements in the primary end point (PFS) or in secondary end points. Safety and tolerability were generally consistent with the respective profiles of zibotentan, carboplatin and paclitaxel. Some potentially promising results from subsets of patients in the zibotentan-treated group were reported but, owing to the small number of patients in this study, these findings should be interpreted with caution and serve as hypotheses that need to be confirmed[108]. Contributory factors to account for the lack of overall clinical benefit in the zibotentan-treated population, and the discrepancies with the encouraging preclinical data, are difficult to identify. One factor might be the lower total dose of chemotherapy received by zibotentan-treated patients relative to placebo-treated patients[108]. In the preclinical models, the treatment was started early in the disease course, when the tumour burden was limited. By contrast, the patients enrolled in this clinical trial had established advanced disease, which, as discussed above, is less affected by zibotentan treatment[108]. Other possible reasons for the disappointing clinical findings could include the lack of predictive biomarkers to identify possible responders. It could also be argued that pharmacological ETAR blockade could tilt the balance towards increased ETBR signalling in the tumour microenvironment. As discussed above, ETBR signalling is pro-angiogenic and may also impair antitumour immunity by preventing the maturation and function of dendritic cells, which are pivotal for the initiation of T cell-mediated immune responses[85,109], and the homing of T cells to tumours[87] (FIG. 4). This could partly explain the failure of specific ETAR antagonists to produce significant clinical results in tumours in which the inhibition of an antitumour immune response might affect overall survival. It is also unclear whether any immunosuppressive effects of ETAR antagonists might be different in the immunocompromised mouse strains used in preclinical studies from those in human patients in clinical trials; any differences may have contributed to the discrepancies between the mouse and human data.

In view of the promising activity of dual ETAR and ETBR antagonists in preclinical models of ovarian cancer[30,31], and the well-tolerated toxicity profile, these small molecules might be considered good candidates for future therapy for ovarian cancer in combination with chemotherapy, although such combinations have not yet shown efficacy in clinical trials in patients with melanoma or glioblastoma (TABLE 2).

Conclusions and future directions

As discussed throughout this Review, the effects of ET1 and its receptors on tumour biology are complex and this might explain why moving agents that target this pathway into the clinic is proving to be a challenge. In solid tumours, the ET1–ETAR axis activates several coordinated and interconnected pathways that also rely on interactions with the tumour microenvironment. Accordingly, therapeutic blockade of ET1 receptors is an attractive approach and has shown anticancer and chemosensitization activity in preclinical studies. However, the outcomes of Phase III clinical trials of this class of small molecules targeting ETAR, despite their manageable side effects, have been somewhat disappointing, and some possible reasons for this are discussed above.

The preclinical data obtained with the dual ETAR and ETBR antagonists indicate that this class of drugs could be a promising therapeutic option for cancer treatment.

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Table 2 | **Endothelin receptor antagonists in cancer therapy**

Compound	Company	ET1 receptor antagonist	Tumour type	Clinical development	Outcome
Zibotentan	AstraZeneca	Specific for ETAR	Non-metastatic CRPC[106]	Phase III (monotherapy)	• OS HR = 1.13; (95% CI = 0.73–1.76) • PFS HR = 0.89; (95% CI = 0.71–1.12)
			HRPC with bone metastases[144]	Phase III (monotherapy)	• OS = 24.5 versus 22.5 months • PFS = 6.2 versus 6.5 months
			Metastatic HRPC*	Phase III (zibotentan plus docetaxel)	• OS = 20.0 versus 19.2 months • PFS = 7.0 versus 7.9 months
Zibotentan	AstraZeneca	Specific for ETAR	NSCLC[145]	Phase II (zibotentan plus pemetrexed)	• OS = 146 versus 193 days • PFS = 110 versus 87 days
Atrasentan	Abbott	Selective for ETAR	Non-metastatic HRPC[146]	Phase III (monotherapy)	• TTP = 764 versus 671 days • OS = 1,477 versus 1,403 days
			Metastatic HRPC[147]	Phase III (monotherapy)	• TTP HR = 0.89 (95% CI = 0.76–1.04) • OS HR= 0.97 (95% CI = 0.81–1.17)
			Metastatic HRPC‡	Phase III (atrasentan plus docetaxel plus prednisone)	NR
YM-598	Astellas Pharma	Selective for ETAR	Prostate cancer§	Phase II (monotherapy)	NR
			Metastatic prostate cancer‖	Phase II (YM598 plus mitoxantrone plus prednisone)	NR
Bosentan	Actelion	Dual ETAR and ETBR	Metastatic melanoma[148]	Phase II (monotherapy)	SD = 34%
			Metastatic melanoma[149]	Phase II (bosentan plus dacarbazine)	• OS = 13.0 versus 10.6 months • PFS = 1.6 versus 2.8 months
Macitentan	Actelion	Dual ETAR and ETBR	Recurrent glioblastoma¶	Phase I (macitentan plus temozolomide)	NR

CI, confidence interval; CRPC, castration-resistant prostate cancer; ET1, endothelin 1; ETAR, endothelin A receptor; ETBR, endothelin B receptor; HR, hazard ratio; HRPC, hormone-refractory prostate cancer; mCRPC, metastatic CRPC; NR, not reported; OS, overall survival; PFS, progression-free survival; SD, stable disease; TTP, time to disease progression.*ClinicalTrials.gov identifier: NCT00617669. ‡ClinicalTrials.gov identifier: NCT00134056. §ClinicalTrials.gov identifier: NCT00050297. ‖ClinicalTrials.gov identifier: NCT00048659. ¶ClinicalTrials.gov identifier: NCT01499251.

A potential advantage of dual ETAR and ETBR antagonists is that they can target not only cancer cells (which typically express ETAR) but also tumour-associated stromal elements, such as vascular, lymphatic and inflammatory cells and fibroblasts, which all express ETBR.

Given the interconnected signalling network of ET1, it is also important to explore the potential value of combinatorial therapies with signal transduction inhibitors[15,110]. For example, the cross-signalling between EGFR and ETAR in ovarian cancer cells provides a rationale to combine EGFR inhibitors with ETAR antagonists[14,15]. Preclinical studies using combined treatment of zibotentan and the EGFR inhibitor gefitinib demonstrated decreased cell proliferation, invasion and VEGF production, accompanied by increased apoptosis[15]. Therefore, using ET1 receptor antagonists in combination with targeted agents to inhibit distinct components of activated signalling pathways may be a useful future approach.

Currently, there are no validated biomarkers that predict responses to treatment with ET1 receptor antagonists, either alone or in combination with other therapies, hence clinical trials of these agents have not yet stratified patients into subgroups that are most likely to benefit from treatment. Future clinical trials should be carefully designed and conducted to identify predictive biomarkers, which will be mandatory for a critical assessment of the therapeutic potential of ET1 receptor antagonists. These biomarkers may reveal mechanisms of intrinsic and acquired resistance, which will inform the rational co-targeting of multiple networks to overcome compensatory mechanisms of therapy escape.

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Competing interests statement

The authors declare no competing financial interests.

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