

## Endothelins in breast tumour cell invasion

Matthew J. Grimshaw

Cancer Research UK Breast Cancer Biology Group, Guy's Hospital, London, UK

### Address for correspondence and reprint requests:

Matthew Grimshaw  
Cancer Research UK,  
Breast Cancer Biology Group,  
3rd Floor, Thomas Guy House,  
Guy's Hospital, London, UK  
SE1 9RT

Tel: 00 44 20 718 84230

Fax: 00 44 20 718 80919

Email: matthew.grimshaw@cancer.org.uk

### Abstract

Endothelins are a family of small, structurally-related, vasoactive peptides that have a great number of physiological roles in many tissues. The 'endothelin axis' consists of three 21 amino acid peptides (ET-1, ET-2 and ET-3), two G-protein-coupled receptors (ET-RA and ET-RB), and two activating peptidases or endothelin-converting enzymes (ECE-1 and ECE-2). There is increased expression of the endothelin axis in invasive breast cancer compared to the normal breast or non-invasive neoplastic tissue. Endothelin expression is associated with invading regions of tumours in patient biopsies and is more common in tumours with high histological grade and lymphovascular invasion, and there is increased systemic endothelin in patients with lymph node metastases compared to those without lymph node involvement. Stimulation of breast tumour cell lines with endothelins leads to an invasive phenotype *in vitro*. Over-expression of the endothelins and their receptors is insufficient to induce an invasive phenotype in benign cells, yet expression by tumour cells leads to markedly increased invasive ability indicating that endothelins act in concert with other factors - both autocrine and paracrine - including cytokines, matrix metalloproteinases and the activation of tumour-associated macrophages. The association between endothelins, poor prognosis and invasion may mean that the endothelin axis is a valid therapeutic target for the treatment of invasive breast cancer.

This review summarises our current knowledge of endothelins in breast cancer invasion and discusses the potential further directions of such research as well as the possibility of anti-endothelin-based therapy of breast cancer.

**Keywords:** Endothelin, breast cancer, chemotaxis, macrophage, invasion.

## ***1. Introduction***

There is a growing interest in the role of the 'endothelin axis' in cancer [1], and the role of endothelins in breast cancer is now under the spotlight. There is increased endothelin expression in breast tumours compared to non-neoplastic tissue [2, 3], and we recently showed that endothelin expression by breast tumour cells and the tumour-associated macrophages (TAMs) leads to complex interactions such that the tumour cells become more invasive [2, 4, 5]. Endothelin expression is associated with invading regions of tumours in patient biopsies [5], and is more common in tumours with high histological grade and lymphovascular invasion [6].

The mechanism by which endothelins induce an invasive phenotype is complex and not fully understood at present, but involves stimulation of both the tumour cells and TAMs, and modulation of matrix metalloproteinases (MMPs) and cytokines. This review summarises our knowledge of the association between endothelins and invasion in breast tumours, and the possible future directions of such research.

## ***2. The endothelin axis.***

Endothelins are a family of small, structurally-related, vasoactive peptides that have a great number of physiological roles, notably in development and vascular homeostasis [7]. The endothelin axis consists of three 21 amino acid peptides (ET-1, ET-2 and ET-3), two G-protein-coupled receptors (ET-RA and ET-RB), and two endothelin-converting enzymes (ECE-1 and ECE-2) [7].

The three endothelin peptide isoforms - which are highly conserved in human, rat and mouse [8] - derive from three separately regulated genes yet have a similar structure [9]: 21 amino acids with a hydrophobic C-terminus and two cysteine bridges at the N-terminus (figure 1a). The peptide sequence of ET-2 and ET-3 differ from ET-1 by two and six residues respectively [8, 10, 11].

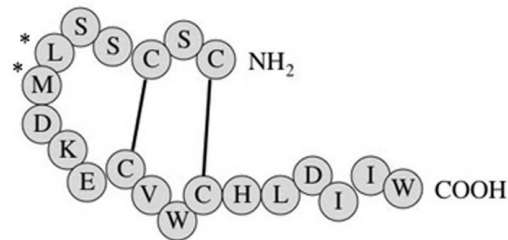
Human ET-1 derives from a 212 amino acid precursor, preproendothelin-1 (figure 1b), which is intracellularly cleaved by the membrane-bound metalloproteinases ECE-1 and -2 [12]. Further enzymes, yet to be identified, are also thought to be involved. Removal of the signal sequence generates the 195 amino acid proendothelin-1, which is further processed to release the intermediate 38 amino acid 'Big ET-1'. Endothelin-converting enzymes hydrolyse Big ET-1 to yield the active 21 amino acid ET-1.

The gene for each endothelin has a distinct pattern of tissue expression: ET-1 is expressed primarily by endothelial cells, ET-2 in epithelial cells of the kidney and intestine, and ET-3 is found in the brain [7]. It is of note, however, that endothelins and their receptors are also expressed by 'mobile' inflammatory cells such as monocytes and macrophages [4, 13]. There is a relatively low basal level of synthesis of endothelins but these genes are readily inducible by inflammatory stimuli such as exposure to cytokines or hypoxia [2, 13].

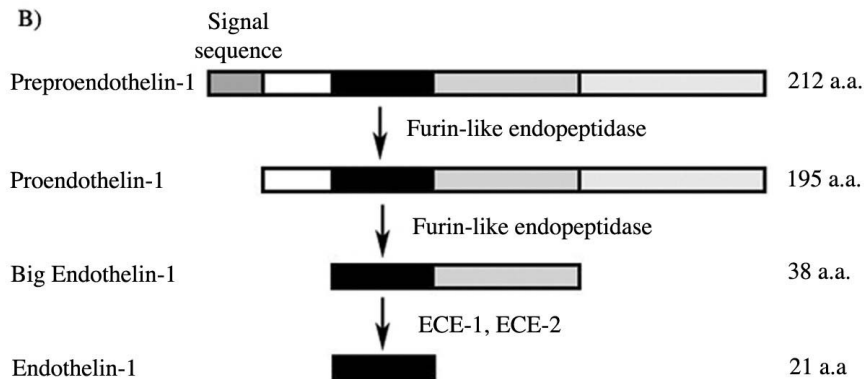
Endothelins bind to two similar receptors, ET-RA and ET-RB [14], with varying affinity: ET-RA binds ET-1  $\geq$  ET-2  $>$  ET-3 but ET-RB shows no selective affinity for any endothelin subtype. ET-RA is expressed by, amongst others, smooth muscle cells, osteoblasts and adipocytes; ET-RB is expressed by all these cells and also by endothelial cells [7]. Binding of the ligands to these G protein-coupled receptors may modulate several overlapping signalling pathways resulting in the activation of phospholipase C

and MAPK pathways, an increase in intracellular calcium and the induction of immediate early genes [1, 15].

A)



B)



**Figure 1.** Processing and structure of endothelin-1. A) ET-1 is a 21 amino acid peptide with a hydrophobic C-terminus and two disulphide bonds at the N-terminus. ET-2 and ET-3 are structurally similar to ET-1, differing by two and six amino acids respectively. The amino acids which differ in the ET-2 sequence are indicated by '\*' B) The 212 amino acid (a.a.) human preproendothelin-1 is processed by proteases to form 'big-ET' intermediates, which are further cleaved to yield the mature endothelin.

### 3. Endothelins in cancer.

Numerous tumours, including carcinomas of the lung [16], prostate [17] and ovary [18] produce one or more of the endothelins and their receptors [1]. Endothelins play an autocrine and paracrine role in regulating growth of several tumour types including ovarian cancer cell lines and tumours [18]; the mitogenic effect of ET-1 in ovarian carcinoma cells is mediated via ET-RA. Endothelins may also inhibit apoptosis [2] and promote angiogenesis [19]. Further, endothelins may be associated with progression and invasion.

However, the actions of endothelins in cancer are from clear and appear to be tumour-specific [20]. In several types of tumour, expression of the endothelin axis - particularly the receptors - is *decreased* in neoplastic tissue. In prostate cancer, ET-RB is decreased or absent [21] and there is often methylation of the ET-RB gene [22], while in lung cancer, ET-RA is down-regulated [23].

### 4. The endothelin axis in breast cancer.

There is increased expression of several members of the endothelin axis in breast cancer. In the normal breast, ET-1 and ET-RA mRNA expression can be detected; however, in

invasive ductal carcinoma (IDC) of the breast there is increased expression of ET-1, ET-2, ET-RA and ET-RB [2, 3]. IDCs also have a higher degree of endothelin expression than either benign fibroadenoma or ductal carcinoma *in situ* (DCIS). Elevated expression of ET-1 is more common in IDCs with larger size, high histological grade and the presence of lymphovascular invasion [24], and there is increased endothelin in the serum of breast cancer patients with lymph node metastases compared to those with no lymph node involvement (Hagemann *et al.* - submitted).

Cells expressing endothelins and their receptors in IDC include the tumour cells [2], the CD68<sup>+</sup> macrophage infiltrate [4], and the endothelial cells [19]. In biopsies of invasive breast cancer, the expression of ET-1, ET-RA and ET-RB is associated with increased vascular endothelial growth factor (VEGF) expression and vascularity [25].

In keeping with the polyfunctional nature of endothelins, there are numerous potential consequences of endothelin expression in breast tumours that may lead to a more aggressive tumour cell phenotype (table 1).

**Table 1.** Potential roles of endothelins in breast tumours, and breast tumour cell invasion.

<b>Role</b>	<b>Cell type</b>	<b>Endothelin and receptor</b>	<b>Notes</b>	<b>Reference</b>
<b>Activation of TAMs</b>	Macrophage	ET-1, ET-2 ET-RB	Foamy activated macrophages colocalise with endothelins <i>in vivo</i> .	[4]
<b>Recruitment of TAMs</b>	Macrophage	ET-1, ET-2 ET-RB	PBMC-derived macrophages chemotax towards ET-1 and ET-2 via ET-RB	[4]
<b>Survival</b>	Breast tumour	ET-2 ET-RB	ET-2 prevents hypoxia-associated apoptosis	[2]
<b>Induction of cytokines</b>	Macrophage Breast tumour	ET-1, ET-2 ET-RA	Endothelins induce TGF- $\beta$ , TNF- $\alpha$ and IL-8.	Hagemann <i>et al.</i> - submitted.
<b>Chemotaxis</b>	Macrophages Breast tumour	ET-1, ET-2 ET-RA, ET-RB	Endothelins induce chemotaxis towards chemokines and tumour cells chemotax towards endothelins.	[2]
<b>Induction of MMP activity</b>	Macrophage Breast tumour	ET-1, ET-2 ET-RA, ET-RB	Numerous MMPs induced by endothelins and TIMP activity reduced.	[2] Hagemann <i>et al.</i> - submitted.
<b>Induction of VEGF</b>	Breast tumour Endothelial	ET-RA	Endothelin expression is associated with VEGF expression and microvessel density.	[25]
<b>Induction of invasion</b>	Macrophage Breast tumour	ET-1, ET-2 ET-RA, ET-RB	Invasion induced, via both receptors, in a multi-factorial pathway.	[5]
<b>Intravasation, extravasation</b>	Macrophages Breast tumour Endothelial VSMC	ET-1, ET-2 ET-RA, ET-RB	High levels of endothelins in the vasculature may induce invasion through the vascular wall.	

### **5. Endothelins and the immune infiltrate.**

High levels of macrophage infiltrate in breast tumours correlate with a poor prognosis [26]. TAMs are able to promote tumour growth directly by secreting breast tumour mitogens, such as epidermal growth factor, and indirectly by stimulating tumour angiogenesis and metastasis [27]. Macrophages produce endothelins [13] and the TAMs contribute to the endothelins in the breast tumour microenvironment [4]. In contrast, no immunoreactive endothelin can be detected in cell extracts from human neutrophils and lymphocytes [13]. Co-culture of tumour cells with macrophages leads to increased endothelin production (Hagemann *et al.* - submitted), Macrophages express both endothelin receptors and chemotax towards endothelins via ET-RB and a pertussis-toxin sensitive MAPK-mediated signalling pathway [4]. Exposure of macrophages to endothelins *in vitro* leads to increased cell surface CD68 and HLA-ABC indicating an 'activated' phenotype, whilst in patient biopsies 'foamy' activated macrophages co-localise with regions containing tumour cells that express endothelins [2, 4].

### **6. Endothelin expression and the breast tumour microenvironment.**

The expression of endothelins is increased in unstimulated tumour cells compared to benign cells (Hagemann *et al.* - submitted); which aspect of transformation leads to increased endothelin expression is as yet unknown. However, the levels of endothelin synthesis by tumour cells may be stimulated by numerous factors that are present within the breast tumour microenvironment. Solid tumours do not consist of a homogeneous structure or environment: one region of tumour, compared to another, may differ in the levels of hypoxia, cytokine concentration, immune infiltrate, vascularisation, necrosis *etc.* [28-30]. The breast tumour microenvironment, particularly hypoxia, modulates expression of numerous 'pro-tumour' genes [31], including those of the endothelins and their receptors [2].

*In vitro*, hypoxia increases expression of ET-2 and both receptors by breast tumour cells leading to autocrine protection from hypoxia-associated apoptosis [2]. *In vivo*, endothelin expression co-localises with areas of hypoxia in a murine model of breast cancer, and the ET-RB antagonist BQ788 [32] increases the development of necrosis [2]. Induction of endothelin expression by hypoxia is via the transcription factor hypoxia inducible factor (HIF) 1 $\alpha$  [33]. Conversely, endothelins stabilise the HIF1 $\alpha$  transcriptional complex leading to expression of angiogenic molecules such as VEGF [34]; accumulation of the HIF1 $\alpha$  subunit is associated with breast cancer progression [35].

As well as hypoxia, soluble factors such as cytokines modulate the expression of the endothelin axis in breast tumours. Of particular importance is the finding that endothelins themselves stimulate endothelin receptor production by breast tumour cells [5].

Within the vasculature, ET-RA is upregulated in smooth muscle cells by insulin and nitric oxide, while in endothelial cells ET-RB is upregulated by TNF- $\alpha$  and bFGF. In the PC3 human prostate cancer cell line, ET-1 is up-regulated by IL-1 $\beta$ , TNF- $\alpha$ , and TGF- $\beta$  [36]. Macrophage-derived cytokines and chemokines, including CCL2, CCL3 and CCL5, induce endothelial cells to produce endothelins [37, 38] which may further modulate the local endothelin concentrations in the tumour microenvironment.

### **7. Endothelins and breast tumour cell invasion.**

Invasive capacity is modulated by endothelins in ovarian carcinoma cells [39], Ewing's sarcoma and neuroblastoma cells [40] and the case for endothelins having a role in breast cancer invasions is compelling:

- i) There is increased expression of endothelins in IDC compared to normal or benign breast tissue, or to DCIS [2, 3].
- ii) Endothelins are increased in the serum of breast cancer patients with lymph node metastases compared to those with no lymph node involvement (Hagemann *et al.* - submitted).
- iii) ET-RA expression by tumours is associated with the incidence of distant metastases and local recurrence [24].
- iv) The expression of endothelins and the receptors (particularly ET-RB) by tumour cells is highest in invading regions of the tumour and in corresponding lymph node metastases [5].
- v) Exposure of tumour cells to endothelins leads to an invasive breast tumour cell phenotype *in vitro* [5].

Invasion of breast tumour cells is thought to be promoted by endothelins via several different (autocrine and paracrine) mechanisms including the modulation of MMP activity, induction of pro-invasive cytokines and inhibition of anti-invasive cytokines.

*In vitro*, the invasive capacity of breast tumour cell lines correlates with the level of expression of the endothelin axis (particularly ET-1), and over-expressing endothelins and/or endothelin receptors leads to increased invasion of the MCF-7 breast tumour cell line (Hagemann *et al.* - submitted).

MCF-7 cells transfected with ET-expressing vectors increase endothelin production several hundred-fold and the basal levels of invasion increase approximately five-fold for ET-1 and ET-2 over-expressing cells and three-fold for ET-3 over-expressing cells (Hagemann *et al.* - submitted).

Transfection of the endothelin receptors alone into tumour cells does not give rise to increased basal invasion of unstimulated cells, but tumour cells are able to respond to diminished levels of endothelins (either added exogenously or produced in macrophage/tumour cell co-culture) and increase invasion compared to mock-transfectants (1-10 ng/ml ET compared to 100 ng/ml ET) (Hagemann *et al.* - submitted). However, the greatest level of invasion is induced by transfecting cells with endothelins as well as the receptors. When tumour cells are co-transfected with vectors expressing either receptor as well as ET-1 or ET-2, basal levels of invasion are markedly increased compared to mock-transfectants (Hagemann *et al.* - submitted). It is interesting to note that while endothelin-mediated invasion of breast tumour cells involves both receptors, transfection of *either one* of the receptors leads to markedly increased invasion. This indicates that tumour cells expressing endothelins and one or more of the receptors will have the highest invasive capacity; *in vivo*, we noted that all tumour cells that stained positively for the endothelin peptides also stained positively for the endothelin receptors, particularly ET-RB [5]. Further, there is autocrine stimulation of tumour cells such that upon exposure to endothelins they increase endothelin receptor production. This positive-feedback loop is likely to drive endothelin-mediated invasion once endothelin expression has been induced by transformation or the tumour microenvironment.

However, expression of the endothelins and their receptors by a benign mammary epithelial cell line, hTERT-HME1, is not sufficient to elicit an invasive phenotype (Hagemann *et al.* - submitted) and it is likely that the endothelins are acting in concert with other factors yet to be elucidated to induce invasion in tumour cells [41].

*In vitro*, stimulation with endothelins induces an invasive phenotype in breast tumour cells and increases invasion through an artificial basement membrane, particularly when co-cultured in the presence of macrophages [5]. The signalling pathways involved in endothelin-mediated induction of invasion of breast tumour cells are yet to be fully described. However, the induction of invasion involves both receptors as both the ET-RA antagonist BQ123 [42] and the ET-RB antagonist BQ788 [32] receptor entirely abolish endothelin-mediated invasion [5]. In single-culture of MCF-7 cells, JNK inhibitors abolish endothelin-mediated invasion yet in co-culture of tumour cells with macrophages, JNK inhibition has only a partial effect (Hagemann *et al.* - submitted). Other inhibitors such as PD98059 (MAPKK inhibitor) and pertussis toxin (G-protein inhibitor) only partially inhibit endothelin-mediated invasion. This indicates that multiple overlapping pathways are activated and that factors in co-culture cooperate with endothelin stimulation to induce invasion.

The increase in invasion stimulated by endothelins is due, at least in part, to increased activity of MMPs: endothelins induce MMP-1, -2, -9 and -14 activity in macrophage culture and MMP-14 activity in MCF-7 culture. TIMP-1 release by macrophages and MCF-7 cells is also reduced by endothelins. Induction of MMP activity is modulated via both receptors and can be inhibited by either BQ123 [42] or BQ788 [32]. The non-selective MMP inhibitor FN439 also blocks endothelin-mediated invasion [5]. In ovarian carcinoma cells, ET-1 binding to ET-RA increases the expression and activation of MMPs whilst decreasing the secretion of tissue inhibitor of matrix metalloproteinases (TIMPs) [39]; unlike breast tumour cells, ET-RB does not appear to be involved in MMP induction in ovarian cancer cells. However, endothelins induce MCF-7 cell invasion with no increase in MMP-1, -2, or -9 activity by the tumour cells; only MMP-14 activity is increased in MCF-7 cell culture (Hagemann *et al.* - submitted). There is little evidence that MMP-14 activity correlates with the invasion of breast cancer cells although a recent publication found that the inhibition of MMP-14 expression may be associated with nm23-mediated inhibition of metastasis in breast carcinoma cells [43]. Therefore, we must conclude that further factors, distinct from the MMPs so far described, are involved in endothelin-mediated breast tumour cell invasion. These findings collectively indicate that endothelins induce invasion via several distinct pathways, including but not limited to the inductions of MMPs, via both receptors and several overlapping signalling pathways.

As well as regulating MMP activity, endothelins modulate the expression and release of cytokines by several tumour cell lines and by macrophages; the data regarding cytokine induction by endothelins and invasion of tumour cells is as yet inconclusive but suggests that endothelins may further modulate the tumour microenvironment and augment invasion of breast tumour cells. Endothelins modulate production of several cytokines by

breast tumour cells and macrophages (Hagemann *et al.* - submitted); ET-1 and ET-2 induce expression of the pro-invasive cytokine TGF- $\beta$  by tumour cells via ET-RA whilst inhibiting expression of the anti-metastatic cytokine IL-10 by macrophages. The induction/repression of cytokines by endothelins is both rapid (3-6 hours) and sustained (>96 hours) in both breast tumour cells and macrophages. In ovarian carcinoma and smooth muscle cells, endothelins induces VEGF production [34, 44] and endothelin expression correlates with VEGF expression in IDC [25]. VEGF promotes breast cancer invasion in an autocrine manner by regulating chemokine receptor expression [45] and this may therefore be another mechanism by which endothelins promote invasion and metastasis.

In the seminal paper by Muller *et al* [46], it was shown that chemokine expression by the organs together with chemokine receptor expression by the tumour cells may lead to the organ-specificity of breast cancer metastases. As well as inducing invasion, ET-1 and ET-2 are also chemotactic for breast tumour cells and this is modulated via both endothelin receptors and a MAPK-mediated pathway [5].

In its role as a chemoattractant for breast tumour cells, it is possible that endothelins may also have a role in breast cancer metastases organ-specificity; however, the physiological concentration of endothelins is below the pharmacological threshold [7] and it seems unlikely that endothelins could direct circulating metastatic tumour cells to specific organs. It is worth noting, however, that several key sites of breast cancer metastases including the brain, bone and lungs all express endothelins and that cells expressing high levels of endothelin receptors respond to low levels (<1 ng/ml) of endothelins.

Further, not only does ET-2 induce chemotaxis of tumour cells towards itself, it also increases chemotaxis towards the chemokines CXCL12 and CCL21 which are involved in breast cancer metastasis [46]. It is possible therefore that the organ-specificity of metastasis of endothelin-stimulated cells is not via the endothelins themselves but by, for instance, CXCL12 released by the proximal lymph nodes.

A further potential implication of the chemotactic nature of endothelins is that they may play a role in lymphovascular intra/extravasation. Endothelial cells produce endothelins (and endothelin expression may be induced by intratumoural hypoxia), which may aid tumour cells to intravasate. The local concentration of endothelins in the vascular wall may be  $\geq 100$  fold that of the plasma level, stemming in part from the fact that 80% of ET-1 is secreted on the basal side of endothelial cells [7]. Tumour cells may be attracted to the vascular wall (either from within the tumour, or circulating tumour cells already within the vasculature) by the high levels of endothelins. Stimulation of tumour cells (and other associated cells such as macrophages) may lead to increased direction-independent invasion as seen *in vitro* [5] and subsequent escape from the tumour into the vascular system (intravasation) or escape into peripheral tissues such as the lymph node (extravasation).

### **8. Therapeutic opportunities.**

The role of endothelins in vasoconstriction has led to the development of small molecule antagonists of the endothelin receptors, which are currently under investigation for the treatment of hypertension, heart failure and renal disease [47]. These antagonists can be administered orally, are well tolerated and have few toxic side-effects.

Atrasentan, a highly selective ET-RA antagonist, has been given to cancer patients in phase II trials for prostate cancer and delayed time to clinical and PSA progression [48]. Phase III trials for hormone-refractory prostate cancer are underway.

In a small scale *in vivo* setting, we treated a murine model of breast carcinoma, HTH-K, with the commercially available modified peptide antagonists of the endothelin receptors, BQ123 [42] and BQ788 [32]. In this study, both antagonists showed a modest survival benefit by two apparently different mechanisms: both caused a delay in growth of the tumour yet BQ123 (ET-RA antagonist)-treated tumours did not show the increased necrosis associated with treatment with BQ788 (ET-RB antagonist) [2]. However, this small study did not examine the effects on invasion or metastases. *In vitro* results suggest that treatment of invasive breast cancer with one or both endothelin receptor antagonists may be of some clinical benefit [5], and pre-clinical trials of such antagonists are presently underway in mouse and rat models of invasive breast cancer.

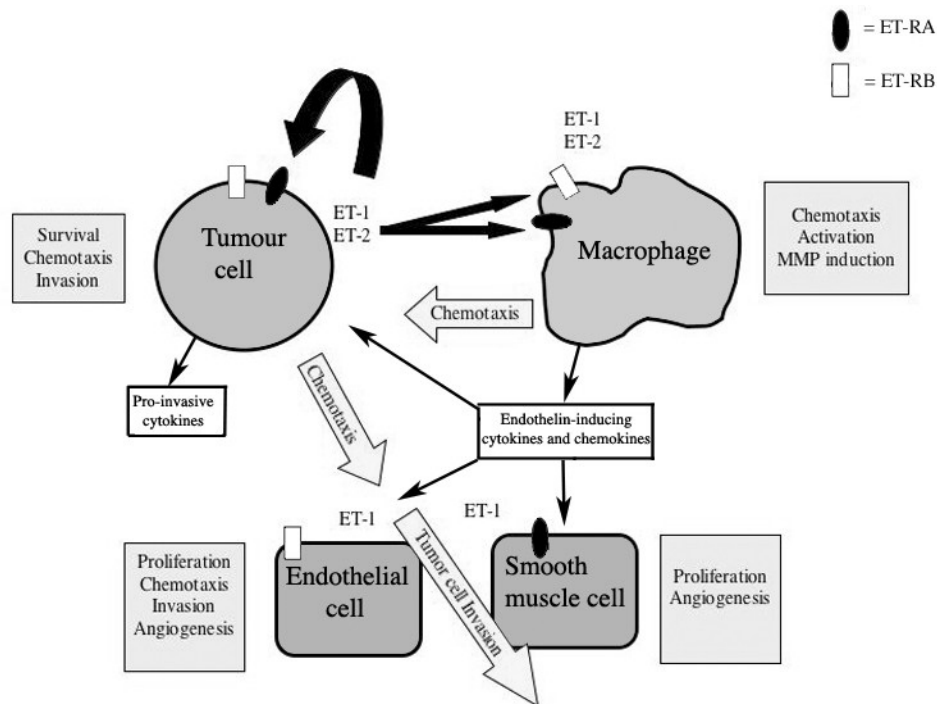
Endothelin receptor antagonists also hold the attractive possibility that they will 'hit' several different cell types and mechanisms of disease progression: the antagonists may potentially inhibit angiogenesis by inhibiting endothelial cell mitogenesis while simultaneously preventing macrophages from producing MMPs, and countering the anti-apoptotic effect of endothelins on the tumour cells themselves.

However, a caveat should be noted: in the HTH-K model, the ET-RB antagonist BQ788 led to increased necrosis [2] which is associated with *increased* invasion and metastasis in patients. It is also of note that the ET-RA antagonist led to 'leaky' vessels that may facilitate metastasis (our unpublished data).

Further, the expression of endothelin receptors by tumours is not associated with progression/invasion in all cancers and thus modifying the endothelin axis may show unpredictable results at secondary sites. For instance, breast tumour cells often metastasise to the lungs; primary tumours in the lung may have decreased ET-RA and hence it is difficult to predict the outcome of treatment with endothelin receptor antagonists on a breast cancer metastasis within the microenvironment of the lung. It is also true that the mechanistic details of endothelin-mediated invasion are yet to be elucidated and so the exact consequences of endothelin receptor inhibition cannot be predicted at this time. Only further research into the endothelin axis in breast cancer and pre-clinical trials of the receptor antagonists will provide an answer as to whether the endothelin receptors are a suitable therapeutic target for breast cancer.

## **9. Conclusions.**

Expression of the endothelins and their receptors is associated with high grade, aggressive breast tumours as well as invasion and metastasis. The mechanism(s) by which endothelins induce an invasive phenotype are as yet incompletely described but potentially includes the interaction between the tumour cells, the infiltrating macrophage and the breast tumour microenvironment. This complex interaction leads to modulation of MMP activity, cytokine expression, immune infiltrate activation, apoptosis and expression of the endothelins themselves. All these factors may cumulatively facilitate breast tumour cell invasion. However, inhibition of either one of the endothelin receptors - for which small molecule inhibitors already exist - inhibits *in vitro* invasion and the endothelin axis may be a suitable therapeutic target for the treatment of invasive breast cancer that can quickly be exploited.



**Figure 2.** Putative roles of endothelins in breast tumour cell invasion. Tumour infiltrating macrophages express inflammatory cytokines that may induce endothelin expression by both tumour cells and macrophages, which release ET-1 and ET-2, and express both receptors. Microenvironmental factors, including hypoxia and the endothelins themselves, further stimulate the endothelin axis. Stimulation of tumour cells and macrophages with ET-1 or ET-2 leads to chemotaxis of these cells, induction of MMP activity and cytokine expression, and invasion of the tumour cells. ET-2 also protects tumour cells from apoptosis and activates macrophages. Endothelial cells and vascular smooth muscle cells (VSMC) express ET-RB and ET-RA respectively. Stimulation of endothelial cells and VSMC with endothelins may stimulate angiogenesis.

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