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Permissive role of endothelin receptors in tumor metastasis

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Abstract

Metastasis remains the major driver of mortality in patients with cancer. The multistep metastatic process requires the concerted actions of several genes and involves tumor cell invasion, epithelial mesenchymal transition (EMT), shedding from primary tumor, intravasation, arrest, extravasation and colonization at a preferential site. Understanding this complex process would provide the basis for the development of molecularly targeted therapeutics aimed at the tumor cell or its interaction with the host microenvironment. The neuropeptide hormones endothelins (specially, ET-1) have been correlated with invasiveness and metastasis of several cancers and high ET-1 levels are associated with decreased disease-specific survival. The mechanism(s) by which ET-1 promotes metastasis are being gradually unraveled. Through preferential binding to cognate receptors (ET_AR or ET_BR), ET-1 triggers autocrine and paracrine signaling cascades in tumor, immune and stromal cells, at both primary and distant sites, supporting cancer progression and metastasis. In this review, we will summarize the role of the ET axis in metastasis of different cancers and potential targeting of ET receptors in the therapeutic setting.

Keywords

Endothelin; Metastasis; Inflammation

Introduction

ET-1, an endothelial cell-derived vasoconstrictor peptide, is an important member of the endothelin axis with myriad developmental, physiological and pathological functions (Kedzierski and Yanagisawa, 2001; Herrmann et al., 2009, 2007; Hagemann et al., 2007). The “endothelin axis” consists of three similar small peptides, ET-1, ET-2 and ET-3, two G-protein-coupled receptors, ET_AR and ET_BR, and two membrane-bound proteases, the ET-converting enzymes, ECE-1 and ECE-2 (Kedzierski and Yanagisawa, 2001), that

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

Appendix A. Supplementary data

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activate the secreted pro forms of the peptide. ET-1 production is stimulated by a variety of cytokines and growth factors, hypoxia, and shear stress, while ET_AR activation triggers signaling networks involved in cell proliferation, new vessel formation, invasion, inflammation and metastatic spread (Kedzierski and Yanagisawa, 2001; Bagnato et al., 2005; Giaid et al., 1990; Rosano et al., 2003, 2007a). ET-1 has been shown to activate the pro-inflammatory transcriptional factors AP-1 and NFκB in human monocytes and cancer cells and to stimulate the production of inflammatory cytokines IL-6, CCL2/MCP-1 and COX2, as well as matrix metalloproteinases (MMPs) activity, the key orchestrators of inflammation-mediated cancer cell invasiveness and metastasis (Kandalaft et al., 2009; Sutcliffe et al., 2009; Spinella et al., 2004a, 2004b). Moreover, elevated expression of ECE-1, ET-1 and its receptors have been detected in a variety of malignancies including prostate, ovarian, breast, melanoma, HNSCC, colorectal and bladder cancers (summarized in Bagnato et al. (2011)). In addition to their direct contribution to tumor growth and metastasis, members of the endothelin axis indirectly modulate tumor–host interactions in various milieus' furthering tumor progression and metastasis. For example, ET-1 promotes autocrine/paracrine interactions between fibroblasts and cancer cells in prostate and HNSCC cells (Dawson et al., 2004) and modulates trafficking, differentiation, and activation of tumor-associated immune cells, possibly contributing to immune evasion and resistance to immunotherapy (Kandalaft et al., 2009; Grimshaw et al., 2002; Buckanovich et al., 2008; Said et al., 2011). ET-1 can induce expression of IL-6, CCL-2, as well as MMP and COX-2 activity, key orchestrators of inflammation-mediated cancer cell invasiveness and metastasis via AP-1 and NF-κB (Rosano et al., 2007a, 2001, 2007b; Sutcliffe et al., 2009; Grimshaw et al., 2002, 2004; Said et al., 2011; Browatzki et al., 2007; Spinella et al., 2007). Recently, we reported that tumor ET-1 triggers inflammation in the lung soon after the cancer cells lodged at this site and thus sets up a vicious cycle wherein inflammatory cells would enhance and facilitate the process of metastatic colonization (Said et al., 2011) (Fig. 1).

Ovarian cancer

Ovarian cancer arises from the surface of the ovary. Hence cancer cells shed from the primary tumor can disseminate as floating single cells or spheroids in the peritoneal fluid and lead to implants on the mesothelial lining of the peritoneal cavity or deeply penetrate the omentum. In the ovarian cancer microenvironment, high ET-1 levels were detected in the peritoneal fluid in patients with malignant ascites (Bagnato et al., 2005). In vivo analysis of the ET-1 axis demonstrated a higher expression of ET-1 and ET_AR in primary and metastatic tumors than in normal ovarian tissues. Interestingly, ET-1-producing cells also expressed functional ET_AR, but not ET_BR, indicating that in ovarian tumor cells, ET-1 likely acts as an autocrine factor selectively through the ET_AR (Bagnato et al., 2005). In human ovarian tumors, ET-1 axis overexpression is associated with ascites formation, malignant progression, advanced tumor stage, and enhanced tumor angiogenesis (Bagnato et al., 1999, 2005). Here the ET-1/ET_AR axis drives multiple signaling networks involving epidermal growth factor receptors, integrins, Wnt, and MMPs (Bagnato and Rosano, 2007) that regulate cell survival, invasiveness, angiogenesis, vascular permeability and EMT (Rosano et al., 2010). ET-1 axis has been shown to be regulated by and induce activation of hypoxia-inducible factor 1α (HIF-1α) and EMT transcription factors as β-catenin/TCF, SNAIL and

SLUG, suggesting that the ET-1 axis contributes to the complex cooperation between the intracellular signaling pathways and extracellular signals triggering EMT (Bagnato et al., 2005; Spinella et al., 2004a, 2004b, 2007; Rosano et al., 2001, 2007b, 2010; Bagnato and Rosano, 2007). Genome-wide expression profiling of ovarian carcinoma identified ET_AR as a key gene related to chemoresistance (Rosano et al., 2010). Interestingly, paclitaxel and platinum resistant ovarian cancer cells showed phenotypic changes consistent with EMT, providing strong evidence linking ET_AR signaling pathways to chemoresistance, EMT and stemness of ovarian cancer cells (Rosano et al., 2010).

In ovarian cancer, ET_BR has been recently shown to control T-cell homing to tumors and failure of immunotherapy as vaccine failure was associated with poor accumulation of T cells at the tumor site, in spite of detectable systemic antitumor immune response. ET_BR blockade with specific antagonist BQ-788 greatly enhanced the efficacy of vaccines in both preventing recurrence and therapy directed at established tumors. BQ-788 did not increase systemic immune response to the vaccine in vivo, but rather greatly enhanced T-cell infiltration in tumors following vaccine (Kandalaft et al., 2009). Activation of a paracrine ET-1/ET_BR axis was also found between tumor cells and endothelial cells, whereby tumor cells overexpress and release ET-1 whereas the latter express ET_BR. This axis suppresses T-cell homing (even in the presence of tumor inflammation), and can be disrupted by ET_BR blockade, which in vivo markedly enhances tumor immune therapy (Kandalaft et al., 2009). ET_BR blockade is likely to have also direct antiangiogenic effects through suppression of endothelial nitric oxide. Interestingly, ET_BR upregulation predicts poor outcome in ovarian cancer (Buckanovich et al., 2008).

Bladder cancer

Gene expression data from several independent studies of human bladder cancer have revealed that *ET1* and ET_AR were overexpressed in muscle invasive disease and that the degree of expression is associated with reduced patient survival (Said et al., 2011). This was confirmed at the protein level by ET-1 immunohistochemistry in human bladder cancer tissue microarrays (Said et al., 2011). Interestingly, ET-1 expression was positively correlated with the proinvasive and proinflammatory mediators IL-6, CCL2, COX2 as well as MMP2 and MMP9. However, an apparent paradox that has arisen in the field regarding the role of ET_AR in cancer is the finding that pharmacologic blockade of this receptor has minimal effect on primary tumor growth (Titus et al., 2005; Wulfing et al., 2005a, 2005b) yet affects experimental metastasis (Said et al., 2011; Titus et al., 2005) and that clinical trials with ET_AR inhibitors in patients with advanced cancers appear to have minimal effect (Bagnato et al., 2011; Nelson et al., 2008; Ohlmann et al., 2011; Clezardin, 2011; Nelson et al., 2011).

To decipher the role of ET-1/ET_AR axis in bladder cancer metastasis we used a comprehensive model whereby the endothelin axis, via ET_AR, was shown to drive bladder cancer lung colonization by regulating key factors in the microenvironment of disseminated tumor cells (Said et al., 2011). Using combined genetic and pharmacologic approaches in human bladder cancer cell lines in vitro, we reported the role of ET-1 in autocrine regulation of proliferation in bladder cancer cell lines and that ET-1 stimulates migration, invasion,

and proteolytic activity of such cells through ET_AR. In vivo, using human and murine models of experimental and spontaneous lung metastasis, we showed that tumor ET-1 is a paracrine mediator of early metastatic colonization of the lung through triggering an early inflammatory response in the lung characterized by macrophage influx, and production of pro-inflammatory mediators MCP-1/CCL2, IL-6, and COX-2. Interestingly, the IL-6, MCP-1/CCL2, COX-2, and MMPs induced by ET-1 are also known to breach the lung vasculature and enable extravasation of cancer cells on dissemination of these cells to the lungs (Qian et al., 2009; Gupta and Massague, 2006; Gupta et al., 2007; Mantovani, 2009). This ET-1 or ET_AR mediated early inflammatory response led to subsequent development of macroscopic lung metastases. Interestingly, pharmacologic inhibition of ET_AR by ZD4054 prior to injection of tumor cells reduced the early inflammatory response and subsequent development of lung metastases. In contrast, when administration of ZD4054 was initiated after establishment of the early inflammatory response, the reduction in lung inflammation and clinical lung metastases was less dramatic. We also showed that the cellular effectors of this ET-1/ET_AR axis are likely macrophages since metastasis was suppressed by macrophage depletion by liposomal clodronate nanoparticles. Given this data we speculate that in clinical trials where pharmacologic inhibition of ET_AR did not affect primary tumor growth or established high-volume metastatic disease, these tumors were no longer dependent on macrophages for their maintenance or growth.

Breast cancer

Elevated expression of ET_AR in primary breast carcinoma is associated with reduced disease-free survival time (Wulfing et al., 2003). ET_AR-positive carcinomas frequently show staining for ET_AR in fibroblasts suggesting the existence of a paracrine axis since breast cancer cells express ET-1 (Wulfing et al., 2003; Kojima and Nihei, 1995). High ET-1/ET_AR-positive tumors were associated with clinicopathological markers of aggressiveness and poor prognosis such as tumor size, poor differentiation, high grade, Her-2/neu overexpression, lymphovascular invasion, inflammation as well as with incidence of local recurrence or distant metastasis (Wulfing et al., 2003). The above findings suggested that ET-1 and ET_AR may be useful prognostic biomarkers in breast carcinomas and may help to identify patients who may benefit from adjuvant therapy (Wulfing et al., 2003). Mechanistic studies revealed that endothelins (1 and 2) induce breast cancer cell invasiveness through HIF1α, inflammatory cytokines/chemokines, and MMPs (Grimshaw et al., 2004; Grimshaw, 2005; Yamashita et al., 1995; Wilson et al., 2006; Hagemann et al., 2005). ET-1 secretion in breast cancer cells is induced by EGF via EGFR and HER2 and involves MAPK-dependent signaling. In turn, an ET-1/ET_AR-dependent regulation of EGFR protein expression and phosphorylation (at Tyr845) was observed and conferred an additional anti-proliferative and anti-invasive effect to ET_AR blocker atrasentan, in trastuzumab treated cells (Fischgrabe et al., 2010). ET_AR antagonism also has additive antitumor activity in breast cancer cells treated with aromatase inhibitors in vitro and in vivo (Smollich et al., 2010). ET_AR blocker, ZD4045 was equipotent to aromatase inhibitors and in combination, exerted an additive effect on in vitro cells and in vivo in tumor xenografts (Smollich et al., 2010).

Recently, ET-1 was found to mediate the invasive properties of triple-negative breast cancer (TNBC) (Ha et al., 2011) as a downstream effector of lactoferrin (Lf) which efficiently

downregulates levels of ER-alpha, PR, and HER-2 in a proteasome-dependent manner (Ha et al., 2011) with subsequent loss of responsiveness of breast cancer cells to ER- or HER-2-targeted therapies. Lactoferrin-induced increased invasion of breast cancer cells was mediated via transcriptional stimulation of ET-1 and was effectively blocked by antagonists of the ET-1 receptor. Co-overexpression of lactoferrin and ET-1 in tumors as well as elevated circulating levels was observed in serum from TNBC as compared with samples from ER-, PR-, and HER-2-positive breast tumors. Thus targeting Lf–ET-1 axis in TNBC represents a new promising approach (Ha et al., 2011).

Prostate cancer

ET-1 plasma concentrations are elevated in men with metastatic and hormone refractory prostate cancer compared to those with locally confined disease or healthy control (Nelson et al., 1995). Immunohistochemistry revealed that primary prostate cancers and prostate cancer bone metastases were usually positive for ET-1 expression (Nelson et al., 1995, 1996). Prostate cancers express higher ET-1 and ET_AR but less ET_BR than normal prostate tissue (Nelson et al., 1996). The in vitro mitogenic effect of exogenous ET-1 on prostate cancer cell lines is modest, but it enhances the effects of a variety of growth factors such as basic fibroblast growth factor b (bFGF), insulin-like growth factor (IGF), and platelet-derived growth factor (PDGF) (Nelson et al., 2003), and in conjunction with VEGF, plays a major role in tumor angiogenesis. ET-1, through ET_AR, transactivates EGFR (Konety and Nelson, 2001), a finding that was verified in a recent study by Wang et al. (2011). The ET-1/ET_AR-EGFR pathway was shown to activate the PI3K/AKT pathway, which is known to play an important role in prostate cancer progression (Nelson et al., 2003). ET-1 also activates focal adhesion kinase (FAK) and elevates intracellular calcium in several prostate cancer cell lines (Dawson et al., 2004, 2006; Nelson et al., 2003). ET-1 expression is also associated with the transition from androgen-dependent to androgen-independent disease (Nelson et al., 1995, 1996). Increased levels of ET-1 are also produced by upregulation of ECE-1 in tumor associated stromal cells (Dawson et al., 2004, 2006).

Colorectal cancer

Preoperative plasma big ET-1 concentration is a predictor of overall survival in patients with colorectal cancer suggesting its utility in selecting high-risk, lymph node-negative patients for adjuvant therapy (Elahi and Everson, 2004; Arun et al., 2002, 2004; Hoosein et al., 2007; Lloyd et al., 2011). In another study, however, elevated serum ET-1 levels in patients with colorectal cancer did not seem to be of prognostic value for survival (Peeters et al., 2000). Immunohistochemistry and immunoelectron microscopy demonstrated increased ET-1 expression in colorectal cancer including endothelial and stromal cells within and surrounding primary and liver metastases (Shankar et al., 1998; Simpson et al., 2000). Patients with colorectal cancer with and without liver metastases had elevated plasma levels of ET-1 (Elahi and Everson, 2004; Shankar et al., 1998). Portal vein chemotherapy with ET_AR antagonist BQ123 in a rodent model (Elahi and Everson, 2004; Asham et al., 2001) produced a significant reduction in micrometastasis specifically when given at the time of, but not after, tumor cell inoculation suggesting the role of ET-1/ET_AR in promoting tumor implantation and the initial establishment of micrometastases (Asham et al., 2001). The

frequent overexpression of the ET-1 gene in human colon cancers was reported to be a direct consequence of genetic alterations of β-catenin signaling in these tumors as inactivating mutations in the APC gene or activating mutations in β-catenin lead to the formation of β-catenin/TCF4 complex on the ET-1 promoter, which in turn activates transcription of the gene. ET-1 would contribute to β-catenin's oncogenic program by providing antiapoptotic and growth-promoting functions (Kim et al., 2004).

Head and neck cancer

Activation of the endothelin axis is a feature of head and neck squamous cell carcinoma (HNSCC) (Hinsley et al., 2012). High pretreatment levels of plasma big ET-1 levels were generally associated with posttreatment distant failure in patients with advanced-stage nasopharyngeal carcinoma, (NPC) (Hearnden et al., 2009; Wen et al., 2011). ET_AR was overexpressed in 74% of NPC and its expression was found to be a robust independent determinant of survival and an independent predictor of distant metastasis (Hinsley et al., 2012; Hearnden et al., 2009). Awano et al. (2006) demonstrated that ET-1 is able to act in an autocrine manner to stimulate the proliferation of HNSCC cells via both, ET_AR and ET_BR. In this study, ET-1 was able to stimulate the migration of HNSCC cells, an effect dramatically amplified by the presence of oral fibroblasts. This paracrine stimulation of HNSCC motility results from the ET-1-stimulated proteolytic release of bioactive ligands from fibroblasts, mediated in part by ADAM17, transactivating EGFR on HNSCC cells, triggering an increase in COX-2 expression (Hinsley et al., 2012; Wen et al., 2011; Awano et al., 2006). Interestingly, ET_AR antagonist ABT-627 can inhibit the growth and metastasis of NPC cells and increase sensitivity to chemotherapy (Mai et al., 2006). The EDNRA/H323H polymorphism was found to be an independent prognostic marker for overall survival in patients with locally advanced NPC. Patients with *EDNRA*/H323H had poorer 5-year overall survival compared to patients with wild-type genotype. The functional significance of this polymorphism has yet to be elucidated (Wen et al., 2011).

Melanoma

ET-1, ET-3 and ET_BR are implicated in melanocyte transformation and melanoma progression and ET_BR is the major endothelin receptor expressed by normal and transformed melanocytes (Spinella et al., 2007). Gene expression profiling of human melanoma biopsies and cell lines indicated positive correlation of ET_BR with aggressive phenotype (Imokawa et al., 1992). ET-1 was shown to be secreted by keratinocytes in response to UV, stimulating proliferation, chemotaxis, and pigment production in melanocytes, and promoting melanocyte survival and inhibiting the UV-induced apoptosis through the phosphatidylinositol 3-kinase (PI3K)-Akt pathway (Hachiya et al., 2004; Kadekaro et al., 2005). UV-induced ET-1 via ET_BR down-regulates E-cadherin expression concomitant with increased expression of N-cadherin, MMP-2, MMP-9, and $\alpha_v\beta_3$ and $\alpha_2\beta_1$ integrins and inhibits intercellular communication by inducing phosphorylation of gap junctional protein connexin 43 (Spinella et al., 2007; Jamal and Schneider, 2002). In addition, ET-1/ET_BR upregulates melanoma cell adhesion molecule (MCAM), a marker of melanoma aggressiveness and metastasis, in primary and metastatic melanoma cell lines (Mangahas et al., 2004). In melanoma cell lines, ET increases HIF-1α with subsequent

upregulation of vascular endothelial growth factor (VEGF), COX-1/COX-2 expression and activity and PGE_2 production in normoxic and hypoxic conditions (Spinella et al., 2007). COX-1/COX-2 inhibitors blocked ET-induced PGE_2 and VEGF secretion, MMP activation and cell invasion, indicating that both enzymes function as downstream mediators of ET-1 induced invasive properties. In melanoma xenografts, ET_BR blockade suppressed HIF-1α accumulation, tumor growth, neovascularization, VEGF expression and MMP-2 (Spinella et al., 2007). Moreover, ET-1 and −3 induced secretion of prometastatic CXC chemokines (Mangahas et al., 2005) further implicating the proinflammatory properties of ET-1 in melanoma invasiveness and metastasis.

The endothelin axis in bone metastasis

In addition to the effects of ET-1 on tumor growth and invasiveness, the paracrine effects of tumor-produced ET-1 on bone cells may create a fertile growth environment for tumor cells in bone. In the bone microenvironment, tumor-derived ET-1 stimulates mitogenesis in osteoblasts, which express both ET_AR and ET_BR, and it decreases osteoclast activity and motility (Nelson et al., 2003), and therefore is involved in the formation of osteoblastic lesions that are frequently observed in patients with metastatic prostate cancer and, to a lesser extent, in metastatic breast cancer (Nelson et al., 2003). In preclinical models, osteoblastic bone metastases elicited by human and murine breast cancer cell lines were inhibited by ET_AR antagonist atrasentan (Guise et al., 2003; Yin et al., 2003) and the mixed (ET_AR and ET_BR) inhibitor bosentan (Dreau et al., 2006). The effect of the ET_A receptor-selective antagonist ABT-627 to block ET-1-stimulated osteoblast proliferation and new bone formation was specific because it did not block FGF-2-stimulated new bone formation (Guise et al., 2003). In prostate cancer, ET-1 production was down-regulated by androgens and up-regulated by the bone-associated factors transforming growth factor β (TGFβ), EGF, IL-1-β, IL-1-α, and TNF-α (Le Brun et al., 1999). Co-cultures of prostate cancer and bone have demonstrated that ET-1 production is increased by prostate cancer cells in contact with bone (Chiao et al., 2000). These effects are mediated via ET_AR (Nelson et al., 1995, 1996).

Conclusion

The ET axis is deeply implicated in the malignant process and tumor progression in several tumor types. In addition components of this axis have potential as prognostic and predictive biomarkers. The prometastatic effect of ET/ETRs in most cancers involves paracrine effects regulating tumor-stromal interactions and involving pro-inflammatory cytokines/chemokines, COX2 and the matrix metalloproteinase. Indeed the identification of endothelin and biomarkers associated with it (Said et al., 2011) in primary tumors begets the design of clinical trials with endothelin axis inhibitors either after radical surgery or in combination with standard of care chemo or radiotherapy to hamper metastatic seeding. Interestingly, despite significant evidence for a role in cancer, prostate cancer has been the only human tumor where significant clinical studies have been undertaken to explore the benefits of interrupting the ET axis.

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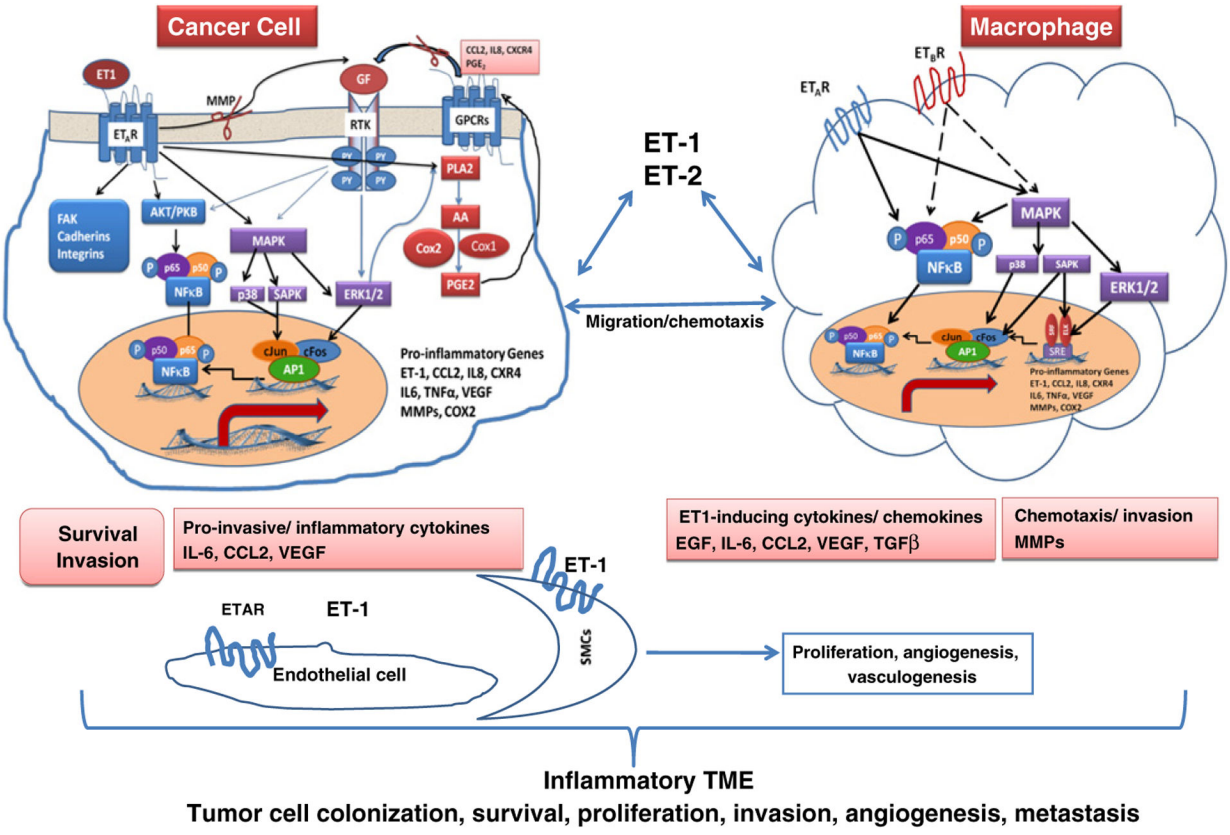

Fig. 1.

Tumor cell colonization, survival, proliferation, invasion, angiogenesis, metastasis. Differential roles of endothelin axis in the tumor microenvironment:

- In tumor cells: Binding of ET-1 to the ET_AR triggers multiple signal-transduction pathways, leading to cell survival and invasion. ET-1 increases its own secretion as well as the secretion of cytokines and growth factors IL-6, CCL2 (MCP-1), and VEGF as well as inflammatory mediators COX2 and prostaglandins-E2 (PGE-2).
- In macrophages: ET-1 induces macrophage chemotaxis through ET_AR and ET_BR. Binding of ET-1 to both receptors in macrophages triggers signaling pathways converging in NFκB and AP-1 activation with subsequent induction of IL-6, CCL2, VEGF, EGF, TGFβ, MMPs and COX2, subsequently, stimulating tumor cell invasiveness, recruitment and colonization in preferential metastatic sites.
- In endothelial cells and vascular smooth muscle cells: ET-1 stimulates endothelial and VSMCs proliferation, angiogenesis and vasculogenesis through interaction with ET_AR receptor.