Minireview

Endothelin-1: a multifunctional molecule in cancer

K Grant,* M Loizidou*1 and I Taylor1

*1Department of Surgery, Royal Free and University College London Medical School, University College London, UK

Endothelin-1 is a small vasoconstrictor peptide that was first identified in 1988. Here we review the evidence implicating ET-1 in tumorigenesis. In particular, we concentrate on the role of ET-1 in mitogenesis, apoptosis, angiogenesis, tumour invasion and metastasis, and discuss the potential for endothelin-system modulation as an adjuvant therapeutic strategy.

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The potent vasoconstrictor peptide endothelin-1 (ET-1) was first isolated from the culture media of porcine endothelial cells in 1988 (Yanagisawa et al, 1988). It is one of a family of multifunctional peptides (ET-1, 2 and 3) that are closely related to the sarafotoxins derived from the venom of the burrowing asp. Of these isoforms, ET-1 has been the most extensively studied to date, and has been implicated in cancer.

ET-1 is synthesised via proteolytic cleavage of a large precursor molecule, pre-pro ET-1, which is facilitated by the metalloproteinase, endothelin converting enzyme (ECE). This pathway is summarised in Figure 1. The endothelins exert their physiological effect via two receptors, ETA and ETB, which are G-protein-coupled transmembrane receptors found in both vascular and nonvascular tissues. Ligand-receptor binding induces dissociation of the receptor-linked G-protein subunits, which may then associate with multiple intracellular effectors. The ETA receptor has varying affinities for the endothelin isoforms (ET-1 > ET-2 > ET-3), whereas the ETB receptor shows no selective affinity for any of the ET subtypes (Sakamoto et al, 1991).

The endothelins have been implicated in numerous physiological and pathological conditions, including hypertension, cardiac failure and disseminated intravascular coagulation. Interest in the role of ET-1 in cancer has grown over the last decade, following the work of Kusuhara et al (1990) that demonstrated ET-1 production by several tumour cell lines. Currently there is evidence that ET-1 may modulate mitogenesis, apoptosis, angiogenesis, tumour invasion and development of metastases. The aim of this article is to review the role of ET-1 in cancer and possible ET-system modulation as an adjuvant therapeutic strategy.

ENDOTHELIN EXPRESSION IN CANCER

Elevated plasma levels of ET-1 have been detected in patients with various solid tumours, including hepatocellular, gastric and prostate cancer (Nakamuta et al, 1993; Nelson et al, 1995; Ferrari-Bravo et al, 2000), where levels are greatest in patients with metastatic, hormone refractory disease.

Our group has demonstrated elevated plasma levels of ET-1 in patients with primary colorectal cancer, with and without liver metastases (Shankar et al, 1998), compared with healthy controls. Plasma levels of Big ET-1 have also been found to be significantly raised in colorectal cancer patients compared with age- and sex-matched controls (Simpson et al, 2000). Of note, this study also found that both preoperative and intraoperative portal plasma levels were significantly higher in Dukes’ D carcinomas compared with localised or regional disease.

Many human cancer cell lines have been shown to synthesise ET-1 in vitro, including colonic, breast, stomach, prostate and glioblastoma cells (Kusuhara et al, 1990; Ali et al, 2000b). This is also reflected in vivo, where increased tissue immunoreactivity for ET-1 has been demonstrated in numerous cancer types, including ovarian, hepatocellular and breast tumours (Bagnato et al, 1999; Yamashita et al, 1991; Suzuki et al, 1998). We have reported increased immunopositivity for ET-1 in colorectal cancer sections. Of note, no correlation was noted between intensity of staining and Dukes’ staging (Asham et al, 2001).

Furthermore, changes in the expression of endothelin system components have also been demonstrated in premalignant tissues. Egidy et al (2000) demonstrated, using reverse transcriptase polymerase chain reaction (RT–PCR), increased expression of pre-pro ET-1 and ECE mRNA in colorectal adenomas compared with normal colon. Also, Alonen et al (2000) demonstrated that immunoreactivity for ET-1 in breast ductal carcinoma in situ (DCIS) specimens was significantly higher (P < 0.005) than that in normal breast tissue. A further significant increase in immunoreactivity was found in invasive tumours compared with DCIS (P < 0.02). These results suggest that modulation of the endothelin system may be an early phenomenon in tumorigenesis.

ENDOTHELIN RECEPTORS AND CANCER

Increased ETA receptor expression in malignant tissue has been demonstrated using immunohistochemistry and/or autoradiography in several cancer types including colorectal, ovarian and prostate tumours (Nelson et al, 1996; Bagnato et al, 1999; Ali et al, 2000a). In the latter, levels of receptor expression have been found...
to correlate with both Gleason score and presence of metastases (Gohji et al., 2001).

Of note, in normal tissue from these sites the ET\textsubscript{B} receptor predominates, whereas the ET\textsubscript{A} receptor becomes prevalent in both primary tumours and metastases. Interestingly, relative hypermethylation of the ET\textsubscript{A} gene has been demonstrated in several prostate, bladder and colon cancer cell lines. Furthermore, this has also been found to correlate with transcriptional down-regulation (Pao et al., 2001), providing a plausible mechanism for reduced ET\textsubscript{B} receptor expression in malignant tissue.

ENDOTHELIN AS A MITOGEN

ET-1 has been shown to stimulate the growth of several human cancer cell lines in vitro including colorectal, ovarian, prostate, Kaposi’s sarcoma and melanoma cells (Yohn et al., 1994; Nelson et al., 1996; Bagno et al., 1999; Ali et al., 2000b; Bagno et al., 2001). Several groups have demonstrated that in epithelial tumours in vitro, this mitogenic effect is mediated via the ET\textsubscript{A} receptor (Nelson et al., 1996; Bagno et al., 1999; Ali et al., 2000b).

The growth of nonepithelial tumours does not appear to be ET\textsubscript{A} dependent. Studies on human melanoma cells have shown that the mitogenic effect of ET-1 is purely ET\textsubscript{B} receptor dependent (Kikuchi et al., 1996), whereas antagonism of either receptor partially inhibits in vitro growth of Kaposi’s sarcoma cells (Bagno et al., 2001). This has also been demonstrated in vivo, where the specific ET\textsubscript{B} antagonist (BQ788) was shown to significantly slow melanoma tumour growth in nude mice (Lahav et al., 1999).

The role of ET-1 as an autocrine growth factor has been demonstrated in human ovarian and colon cancer cell lines (Bagno et al., 1995; Ali et al., 2000a). Furthermore, Moraitis et al. (1999) have implicated ET-1 as a paracrine growth factor in ovarian cancer. They demonstrated that ET-1 production by human ovarian cancer cells stimulated the growth of carcinoma-associated fibroblasts in coculture, an effect that was partially inhibited by both ET\textsubscript{A} and ET\textsubscript{B} antagonism. However, a recent study by Kernochan et al. (2002) found that ET-1 has no effect on human colonic subepithelial myofibroblast proliferation, although contraction and migration of these cells was stimulated through ET receptor-mediated myosin phosphorylation. The effects of ET-1 on proliferation and other cellular processes in cancer are summarised in Figure 2.

ENDOTHELIN AND APOPTOSIS

In addition to its mitogenic effect, there is evidence that ET-1 may also contribute to tumour growth by protecting cells from apoptosis. ET-1 has been shown to protect rat fibroblasts and human endothelial cells (Wu-Wong et al., 1997) from serum-deprivation-induced apoptosis in vitro (Shichiri et al., 1997).

Peduto-Eberl et al. (2000) have also more recently demonstrated that ET-1 is a survival factor for rat colon carcinoma cells against FasL-mediated apoptosis. From these data, it could be suggested that ET-1 may influence tumour growth by influencing both cellular proliferation and cell death.

ENDOTHELIN AND ANGIOGENESIS

Endothelin-1 may also facilitate tumour growth through the promotion of angiogenesis. ET-1 is a potent mitogen for both endothelial cells and vascular smooth muscle cells (VSMC) in vitro (Komuro et al., 1988; Pedram et al., 1997). In addition, ET-1 may indirectly enhance endothelial cell proliferation through stimulation of vascular endothelial growth factor (VEGF) production by other cell types (Pedram et al., 1997; Salani et al., 2000a). The reverse situation has also been demonstrated in endothelial cells, where VEGF has been shown to enhance ET-1 mRNA expression and ET-1 secretion (Matsuura et al., 1998).

Furthermore, ET-1 potentiates the effect of several proangiogenic factors in vitro, including PDGF and VEGF (Pedram et al., 1997; Yang et al., 1999). ET-1 also stimulates invasion and morphological differentiation of human umbilical vein endothelial cells (HUVEC) in matrigel in vitro, and this may be facilitated via ET-1-induced production of matrix metalloproteinase-2 (MMP-2) by endothelial cells (Salani et al., 2000b). In vivo, when combined with VEGF, ET-1 has been shown to stimulate angiogenesis in subcutaneously implanted matrigel plugs in mice (Salani et al., 2000b). Bek and McMillen (2000) demonstrated that ET-1 also stimulated angiogenesis in a rat corneal model with a similar efficacy to VEGF. In this model they found that ET-1-stimulated angiogenesis was inhibited by either ET\textsubscript{A} antagonism, or mixed antagonism with bosentan, but was not affected by the addition of an ET\textsubscript{B} antagonist. These data suggest that ET-1 may be an important modulator of angiogenesis in cancer.

ENDOTHELIN-1 AND TUMOUR PROGRESSION/METASTASES

There is increasing evidence that ET-1 may also influence tumour invasion and metastases. A recent study in human ovarian carcinoma cell lines has demonstrated that ET-1 can regulate the expression of several MMPs, in particular, MMP-2 and MMP-9, and can downregulate tissue inhibitors of matrix metalloproteinases (TIMP) 1 and 2 (Rosano et al., 2001).

ET-1 may also modulate the growth of bony metastases from prostate cancer. In human prostate cancer cells, ET-1 production is enhanced by bone contact, which in turn blocks osteoclastic bone reabsorption (Chiao et al., 2000). This is also reflected in vivo, where Nelson et al. (1999) used an osteoblastic tumour model (WISH – a human tumour derived from amnion) to demonstrate
that tumours transfected to overexpress ET-1 produced significantly more bone growth in nude mice compared with vector only controls.

Furthermore, our group has demonstrated increased immunoreactivity for ET-1 in endothelial cells within colorectal liver metastases compared with surrounding vessels (Shankar et al., 1998), suggesting that ET-1 may be involved in modulation of tumour blood flow, known to be altered in liver metastases.

ENDOTHELIN ANTAGONISM AS A THERAPEUTIC STRATEGY

Several in vivo models have been used to assess the role of endothelin antagonism in tumorigenesis. Work originating from our department using intraperitoneally injected syngeneic MC28 cells in rats demonstrated that ETA antagonism with BQ123 significantly reduced hepatic tumour load compared with controls (Asham et al., 2001).

Peduto-Eberl et al (2000) assessed the effect of bosentan, a dual receptor antagonist, on the growth of peritoneal tumours derived from a syngeneic rat colon adenocarcinoma cell line. Although bosentan was not able to control tumour progression, they did find that tumours were generally of lower grade, and there were fewer spontaneous deaths in the treated vs the untreated groups. Egidy et al (2000) used the same tumour model to assess histological differences between tumours of bosentan-treated animals and controls. They demonstrated that tumour cells were less densely packed, and there was less collagen matrix around tumour nodules in the treated compared to the untreated group.

Finally, using an osteoblastic tumour model in nude mice Nelson et al (1999) have shown that ETA antagonism with A127722 significantly reduced the growth of new bone compared with vehicle treated controls. Although in vivo results have so far not yielded dramatic results, they are encouraging and warrant further investigation.

Recently, a phase I trial of the ETA receptor antagonist atrasantan was undertaken in 31 patients with refractory adenocarcinomas (Carducci et al., 2002). Nearly half of the patients had prostate cancer (n = 14), although patients with other malignancies, including colorectal (n = 6), breast (n = 2), lung (n = 4) and renal cell carcinoma (n = 3), were recruited. Side effects relating to the physiological consequences of ETA blockade include headache, hypotension and peripheral oedema that were generally tolerated, being mild to moderate in nature. Of the 24 patients who completed the initial 28-day trial, no complete or partial radiological responses were observed. However, a third of patients with tumour-related pain experienced alleviation of symptoms. Additionally, prostatic specific antigen (PSA) levels were found to fall in half of the prostate cancer patients, and reduction in other biochemical tumour markers such as CEA and CA125 were also recorded, suggesting antitumour activity. It remains to be seen whether this will result in a significant clinical benefit.

CONCLUSION

Components of the endothelin system are altered in cancer, and appear to aid tumour growth and progression in a number of epithelial cancer types, via direct and indirect mechanisms. From the evidence to date, it appears that selective ETA antagonism provides the most likely effective method of endothelin system inhibition in cancer. With generally mild to moderate side effects, and suggested antitumour activity, further development and clinical evaluation of these agents is warranted to determine possible therapeutic potential as an adjuvant anticancer strategy.

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