and Big ET-1 levels, and tissue expression of ET-1 in patients with ductal carcinoma of the breast. Methods: Peripheral venous blood samples were collected prior to diagnostic biopsy from women with suspicious non-palpable mammographic lesions. Plasma ET-1 and Big ET-1 levels were determined in 30 patients with IDC, 30 with DCIS and 30 with benign lesions (controls), by performing ELISA. ET-1 and VEGF tissue expression was immunohistochemically determined. Potential correlations with histological grade, hormone receptor status, Her2/neu amplification, tumor size, lymph node involvement and disease stage were investigated in IDC. Results: Big ET-1 plasma levels were significantly higher in IDC and DCIS patients compared to controls (p respectively). No significant differences in ET-1 levels were observed between the three groups. Moderate to strong IHC staining for ET-1 was observed in 3/29 and 7/23 IDC and DCIS patients, respectively. VEGF was significantly expressed in 8/27 and 8/23 IDC and DCIS patients, respectively. In IDC, plasma and tissue expression of ET-1 and ETA plasma expression of ET-1 did not correlate with any of the analyzed clinicopathological characteristics or VEGF tissue expression. Conclusions: Plasma levels of Big ET-1 were a more sensitive indicator of ET-1 deregulation than those of ET-1 in our study. Our results support the potential clinical application of Big ET-1 as a breast cancer biomarker.


The localisation and distribution of endothelin receptors in normal and cancer colon tissues: Confirmation by autoradiography, immunohistochemistry and quantum dot targeting

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Background: Endothelin-1 (ET-1) acts via two G-protein-coupled receptors, ETA and ETB. Overexpressed ET-1 and ETA in colorectal cancer (CRC) promote tumour growth and progression. Aim: To investigate (1) ETA and ETB distribution in normal and cancer tissues from patients with CRC and (2) determine ETA and ETB localisation to cell types and tissue structures. Methods: ETA and ETB distribution was determined using in vitro autoradiography with competitive inhibition, using receptor antagonists (BQ123, ZD4054, BQ788) on normal and cancer tissues resected from patients with CRC (N = 8). Immunohistochemistry (IHC) confirmed ETA and ETB expression and identified associated cells/structures. ETA distribution was also investigated by quantum dots (QDs) conjugated to BQ123 (ETA-antagonist). Results: In normal bowel epithelium, ETA was observed closer to the luminal surface and ETB towards the muscularis mucosa/lamina propria. There was greater ETA than ETB binding in CRC. Both cancer and normal tissues demonstrated strongest binding to stromal cells, particularly fibroblasts (IHC). QD-BQ123 demonstrated an ETA punctate pattern in stromal areas surrounding epithelial cells; and an ETA increase in CRC compared to normal. Conclusions: ET-1 binds strongly to CRC stromal structures, with ETA greater than ETB, and is consistent with ET-1 signalling contributing to tumourigenesis. Within normal tissue, differential ETA and ETB distribution (luminal versus muscularis mucosa/lamina propria) has not been reported previously. This may relate to trophic, growth arrest and differentiation signalling. This study demonstrates the effective, novel use of receptor-antagonist-conjugated QDs; reveals possible ET-1 roles in normal tissue; and provides further evidence for the potential therapeutic use of ETA antagonists as CRC adjuvant treatment.


Novel molecular pathways by which ETA receptor mediates tumourigenic signals in colorectal cancer: Support for ETA receptor antagonism as adjuvant treatment

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Background: The endothelin A receptor (ETA) mediates tumourigenic signals in colorectal cancer (CRC). The ETA ligand, endothelin-1 (ET-1), stimulates not only cancer cells but also surrounding fibroblasts and may promote the creation of a supporting tumour stroma. Aim: To identify ET-1 regulated genes associated with oncogenic pathways in colonic fibroblasts. Methods: Micro-array analysis following 4 h ET-1 stimulation of colonic fibroblast strains (isolated from patients undergoing resection for CRC, n = 4) identified differentially expressed genes (n = 19) at significant levels. Three were investigated further: COLXI, AML-1, and EGFR (collagen type-XI; acute myeloid leukemia-1; epidermal growth-factor receptor). Quantitative RT-PCR (qRT-PCR) and immunoblotting evaluated AML-1 and COLX expression levels, following treatment with ET-1 and/or receptor antagonists (ETA: BQ123, ZD4054; ETB: BQ788). ETA and ETB regulation of EGFR was investigated by gene silencing (siRNA); these assays and ET-1 regulation of EGFR over 24 h were evaluated by qRT-PCR. Results: ET-1 stimulated expression of AML-1 and COLX at both gene (>1.5-fold; p < 0.01) and protein (p < 0.05) levels; stimulation was inhibited by ETA, but not by ETB, antagonism (AML-1: 75.1–77.1% by BQ123, ZD4054; COLX: 65.1% by ZD4054; p < 0.05). EGFR expression demonstrated a biphasic increase at 4 h and 24 h (3.8-fold; 4.5-fold). Silencing ETA, but not ETB, returned EGFR levels to control. Conclusions: ETA antagonism has potential for targeting oncogenic pathways: AML-1 is linked to c-Jun N-terminal kinase which inhibits apoptosis/promotes proliferation; and abnormal TGF-β (transforming growth-factor-beta) signalling. COLX is linked to CRC tumourigenesis. The ET-1-stimulated biphasic EGFR response and ETA antagonism have not been reported before in CRC. These findings identify mechanisms by which ETA promotes tumourigenesis and support addition of ZD4054 to existing EGFR antagonism therapy.


Serum big endothelin-1 as a clinical marker in canine pulmonary hypertension and tumors

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Background: Endothelin has a stimulating action on the cardiovascular and respiratory systems. Endothelin-1 (ET-1) is a potent vasoconstrictor and also has mitogenic action. In experimental animal models, ET-1 administration increases cardiac output in normal dogs and also induces a pressor response in mildly hypertensive dogs. However, there is no information on the serum ET-1 levels in canine pulmonary hypertension. Aim: To determine the serum ET-1 levels in canine pulmonary hypertension and tumors. Methods: Serum ET-1 levels were measured using an ELISA kit in 17 dogs with idiopathic pulmonary hypertension and 15 dogs with other pulmonary diseases. Results: The serum ET-1 levels were significantly higher in dogs with idiopathic pulmonary hypertension (range: 23.2–115.8 pg/mL) compared to dogs with other pulmonary diseases (range: 1.0–19.8 pg/mL). Conclusion: Serum ET-1 levels are significantly higher in dogs with idiopathic pulmonary hypertension than in dogs with other pulmonary diseases.