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 < 0.001 and p < 0.01, 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 plasma expression of Big 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 ETA and ETB 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 COLXI 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; COLXI: 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. COLXI 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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An unexpected pulmonary hypertensive crisis: Eying the culprit

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A 56-year-old man developed sudden dyspnoea after resection of choroidal melanoma in his left eyeball. Worsening hypoxia required intensive treatment, including percutaneous cardiopulmonary support. On contrast-enhanced computed tomography there was no evidence of either thrombi in the pulmonary arteries or obvious lung diseases. A Swan–Ganz catheter showed increased mean pulmonary arterial pressure and no elevation of pulmonary capillary wedge pressure. These findings were consistent with a diagnosis of pulmonary arterial hypertension. Because reports have described a significant relationship between melanoma and endothelin (ET)-1, we hypothesized that a substantial amount of ET-1 had been released from malignant melanoma cells during resection, thus triggering the pulmonary hypertensive crisis in our patient. The patient fully recovered after intensive treatment and administration of the endothelin receptor antagonist bosentan. The success of bosentan treatment, along with an extremely high level of ET-1 on pathologic analysis, confirmed our hypothesis regarding an increase in plasma ET-1 level – 9.60 pg/mL (normal range < 2.3 pg/mL).


Pharmacokinetics of SPI-1620 in a Phase I, open label, ascending dose study of the safety, tolerability, pharmacokinetics and pharmacodynamics of the endothelin B receptor agonist, SPI-1620, in recurrent or progressive carcinoma

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Objective: The primary objective of the Phase I study was to assess the safety and tolerability of SPI-1620 administered to patients with recurrent or progressive carcinoma who had failed all standard therapy. Secondary objectives were to assess PK and PD profiles of SPI-1620, and to identify the optimum dose of SPI-1620 to be used in future Phase II studies. The pharmacokinetic properties of SPI-1620 will be presented. Methods: Eligible patients received SPI-1620 by intravenous infusion over 1 min in an accelerated dose escalation scheme. SPI-1620 doses ranged from 0.5 μg/m\textsuperscript{2} to 15.1 μg/m\textsuperscript{2}. Serial blood samples were collected from each patient prior to infusion (0 min) and at pre-specified intervals from the start of the infusion. Human plasma samples were analyzed by a validated HPLC–MS/MS method. Descriptive PK parameters were determined by standard model independent methods based on the concentration–time data of each subject. Results & conclusion: The highest concentration of SPI-1620 was achieved by the end of infusion. SPI-1620 C max increased proportionally as a function of SPI-1620 dose while the AUC (0–T) increased in a more than dose proportional manner. The SPI-1620 T 1/2 was short and ranged from 4.38 min to 8.29 min. SPI-1620 had a low systemic clearance and small VD (approximately equal to the intravascular volume).


Endothelin-1-induced β-arrestin signalosome is linked to chemoresistance, EMT and stem-cell like properties in ovarian cancer cells

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The epithelial–mesenchymal transition (EMT) is known to play a crucial role in the aggressiveness of epithelial ovarian cancer (EOC), contributing to chemoresistance and cancer stem cell populations. In this tumor, the endothelin (ET)-1/endothelin A receptor (ETAR) axis, by regulating EMT and invasion, endows EOC cells with an increased chemoresistance. Here we examined whether β-arrestin-1 (β-ar1) can act as a nuclear hub orchestrating nuclear signaling in ETAR-driven EMT and chemoresistance. A significant higher expression of β-ar1 and ET-1/ETAR and the stronger presence of β-ar1 in the nuclear compartment upon ETAR activation are present in chemoresistant cells, compared to sensitive cells. In the nuclei, β-ar1 robustly interacts with β-catenin to form a nuclear complex localized on the ET-1 promoter region, leading to transcription of ET-1, demonstrating that β-ar1 drives the positive inter-regulation of ET-1 itself. This autocrine circuit is involved in β-ar1-driven appearance of EMT features and acquisition of stem-cell like properties. Moreover, at functional level, chemoresistant cells, with high nuclear β-ar1, display higher invasive potential and increased resistance to chemotherapeutic drugs. These effects were inhibited by ET-1 receptor blockade with macitentan, or by β-ar1 nuclear mutant.