Endothelin-1 stimulated gene induction within colon cancer cells and fibroblasts and the effect of Endothelin receptor antagonism on key genes.

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Background: The vasoactive peptide ET-1 (Endothelin-1) contributes to colorectal tumourigenesis. At the cellular level, ET-1 promotes proliferation in cancer cells, via one of its two receptors (ET_A). Additionally, ET-1 activates surrounding fibroblasts and may promote the creation of supporting tumour stroma.

Aim: To identify new genes that were up/down-regulated within cancer cells and fibroblasts post-ET-1 exposure and evaluated ET_A & ET_B receptor antagonism on gene expression.

Methods: Illumina micro-arrays determined differential gene expression post-ET-1 stimulation of 3 colorectal cancer cell lines and 4 human colon fibroblast strains isolated from areas adjacent to colorectal cancers. To confirm expression of genes of interest, we examined time point induction mRNA levels (RT-PCR; real-time RT-PCR). ET_A (Zibotentan, BQ123) and ET_B (BQ788) antagonistic effects were measured at the mRNA and protein levels (Immunoblotting). SiRNA was also used to confirm receptor involvement in regulation of these key genes.

Results: Four-hour ET-1 induction had a significant effect on gene up/down-regulation ($p<0.01 \pm \geq 1.5$-fold) in all cancer cell lines (9 genes) and fibroblast strains (111 genes). We determined expression and effect of receptor antagonism of the following (table 1): (a) In cancer cells: (i) MT1X, maximum at 4hr, reversed by ET_A antagonism; (ii) MMP7, late maximum induction (24hr) (undetectable by 4hr microarray), reversed by ET_A antagonism; (iii)PPP2R5D, no significant up/down regulation, but levels were decreased by ET_A antagonism. (b) In fibroblasts: (i) CTGF and (ii) ADM maximum at 2-4hr, both reversed by ET_A & ET_B antagonism; (iii) STC1, transient up-regulation (1hr) followed by down-regulation (4hrs in-line with 4hr microarray data), reversed by ET_A & ET_B antagonism.

ET-1 Regulated Genes

Gene	Name	Role/Effect
MT1X	Metallothioneins	Pro-proliferation/ migration/invasion, angiogenic
MMP7	Metalloproteinase	Pro-invasion/migration
PPP2R5D	Protein Phosphatase 2 Reg5 Delta	Pro-proliferation/division/migration
CTGF	Connective Tissue Growth Factor	Pro-proliferation/adhesion/migration/angiogenesis
ADM	Adrenomedullin	Pro-proliferation/angiogenesis & inhibits apoptosis
STC1	Stanniocalcin 1	Pro-survival/ mitogenic

Table 1. ET-1 regulated genes and their associated roles in tumourgenesis.

Conclusions: ET-1 stimulates cancer cell and cancer-associated fibroblasts to produce signals that promote cancer growth and formation of tumour stroma. ET_A and ET_B receptor antagonists block a number of these signals. This study provides new evidence for the potential therapeutic use of Endothelin receptor antagonists as an adjuvant treatment for colorectal cancer.