Characterisation of Endothelin-1 binding sites in colorectal cancer and antagonistic action of the Endothelin A receptor antagonist Zibotentan (ZD4054)

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Background: Endothelin-1 (ET-1) acts via two G-protein coupled receptors, ET_A and ET_B. Overexpressed ET-1 and ET_A in colorectal cancer (CRC) promotes tumour growth and progression.

Aim: To investigate the distribution of ET_A and ET_B in patient tissue sections. ET-1 affinity (K_d) and receptor density (B_{max}) was determined in whole tissue homogenates, CRC cell lines and colorectal fibroblasts. In addition the effect of the orally active ET_A specific antagonist Zibotentan (ZD4054) on ET-1 receptor binding (IC_{50}) was evaluated against subtype selective laboratory compounds.

Material and Methods: ET-1 receptor distribution and binding characteristics (K_d; B_{max}) were determined using *in vitro* autoradiography on patient sections, tissue homogenates, CRC cell lines and primary fibroblasts. Immunohistochemistry (IHC) identified stromal structures. Study was awarded ethical approval, REC No. 08/H0720/162, University College London Hospitals

Results: ET-1 binding to cancer and normal colon tissue had similar characteristics. There was greater ET_A than ET_B binding in CRC sections. Both cancer and normal tissues had strongest binding to stromal cells, particularly fibroblasts (IHC). Furthermore, characterising CRC cell lines and primary fibroblasts revealed high density and affinity ET-1 binding (B_{max} 1.11 fmol/1x10^6 cells; K_d 450.5 pmol/L and B_{max} 3.03 fmol/1x10^6 cells; K_d 213.6 pmol/L respectively). Inhibition studies showed ET_A antagonists (BQ123; Zibotentan) more effectively reduced ET-1 binding (approximate IC_{50} values in CRC: 10μM, 0.1μM respectively; fibroblasts: 0.1μM, 10μM respectively) than ET_B antagonism BQ788 (approximate IC_{50}; 1mM in both).

Conclusions: ET-1 binds strongly to CRC stromal structures (fibroblasts; endothelial cells), and is consistent with ET-1 signalling contributing to tumourigenesis. We further demonstrated that the orally active ET_A antagonist Zibotentan reduces ET-1 binding to CRC tissues. This study provides further evidence for the potential therapeutic use of the specific ET_A antagonist Zibotentan as an adjuvant treatment for CRC.

CRC=colorectal cancer; ET-1=Endothelin-1; IHC=Immunohistochemistry.