

## **EVALUATION OF A TRANSFECTED HEK293 CELL LINE OVEREXPRESSING THE GC-C RECEPTOR**

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Guanylate cyclase C (GC-C) is typically highly expressed in the brush border of the intestinal epithelium and in human colorectal adenocarcinomas, but is minimally expressed in extraintestinal tissues. Many studies have demonstrated that GC-C is a useful target for imaging and treatment of colorectal cancers using GC-C ligands. In this study, we have established a transfected Hek293 cell line overexpressing the human GC-C receptor and tested its ligand binding, proliferation, biodistribution and imaging properties.

A pcDNA3.1(+)/GC-C plasmid was constructed and used to stably transfect Hek293 cells. A Hek293/GC-C cell line was successfully selected and confirmed by receptor binding studies, western blot and RT-PCR. Transfection did not significantly influence the in vitro cell growth rate compared with Hek293/control. Scatchard assay, immunoblot, and RT-PCR analyses all demonstrated significant overexpression of GC-C in the transfected cell line, and the functionality of the expressed protein was demonstrated by a > 150-fold increase in generation of cGMP upon ligand stimulation relative to the control cell line. Further, treatment of Hek293/GC-C with GC-C ligands resulted in decreased cell proliferation measured by MTT assay, as has been previously observed for other GC-C-expressing colorectal cancer cell lines. Biodistribution and in vivo imaging studies carried out in nude mice bearing Hek293/GC-C xenografts also demonstrated high specific uptake of an Indium-111-labeled GC-C agonist. The Hek293/GC-C cell line described here will provide a useful model for the development of GC-C agonists as diagnostic and therapeutic agents for colorectal cancer.