

The Effects of CodeBreaker™ Silencing of CG β Genes on Bladder Cancer Cell Line Viability.

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Introduction: Human chorionic gonadotrophin β -subunit is coded for on chromosome 19 by a cluster of six genes/pseudogenes, designated as *CG β 1*, *CG β 2*, *CG β 3*, *CG β 5*, *CG β 7* and *CG β 8*. Previous research has shown the magnitude of *hCG β* gene expression in the placenta differs for each homologue where: *CG β 5* expression is greater than that of *CG β 3* and *CG β 8*, which are in turn greater than the expression of *CG β 7*, *CG β 1* and *CG β 2* respectively. Furthermore, expression of *CG β 1* and *CG β 2* is believed to produce non-functional transcripts due to aberrant splicing of the first exon.

Ectopic expression of free β -hCG by many common mucosal and epithelial cancers is now well recognised and associated with poor prognosis. Moreover, the structure of β -hCG is found to be similar to a family of autocrine growth factors which are also the products of known oncogenes. Recent studies have shown that bladder carcinoma expression of β -hCG is due to expression of genes *CG β 3*, *CG β 5* and *CG β 8* as in the placenta. However, in these studies *CG β 1* and *CG β 2* genes have not been included in the study design as they were dismissed as pseudogenes due to the alternative splicing found in placental tissue studies.

Aims: To transfect SCaBER cell line with siRNAs to *CG β 1&2* and *CG β 3,5,7,8* genes using CodeBreaker™ transfection reagent and examine the effect of the *hCG β genes* on tumour cell survival.

Methodology: In this study *hCG β* mRNA was targeted using short interfering RNAs (siRNAs) specifically designed to silence the translation of mRNA coding sequences arising from *CG β 2* as apposed to *CG β 3,5,7,8* genes. CodeBreaker (Promega), a proprietary formulation optimized for the efficient transfection of short interfering RNA was employed as the transfection protocol and the affects of transfection on cell viability/number was assessed by the MTS assay. Bladder cancer cell line SCaBER was transfected with both siRNA's and MTS determined viability determined after 48hrs in culture. All data was corrected (normalised) against MTS cell viability scores achieved for a negative control of the bladder cancer cell line exposed to a scrambled sequence siRNA at 25nmol concentration. In addition, a second independent negative control of siRNA for EGFP (Enhanced Green Fluorescent Protein) mRNA sequence was also run in every experiment.

Results: Using 0.6 μ L of CodeBreaker™ transfection reagent, SCaBER cells exposed to siRNA for *CG β 1&2* genes showed significant reduction in cell numbers with a maximum decrease of 44% at 25nmols of siRNA (24% for 20nmols of siRNA and 19% for 15nmols). By way of contrast, the second negative control siRNA for EGFP resulted in a 13% decrease in cell numbers. Interestingly, when *CG β 3,5,7,8* were targeted by siRNA using maximum amount of CodeBreaker™ (1 μ L), at 25nmols of siRNA the maximal reduction in cell numbers was only 23%.

Conclusion: Cell culture viability for SCaBER cells is reduced when cultures are transfected with anti *CG β* siRNA. This supports the hypothesis that *hCG β* acts as a growth factor for such tumours. However, the data suggests that *CG β 1&2* are not mere pseudogenes and play an important role in ectopic expression and tumour cell growth.