Identification and functional characterisation of G protein-coupled receptor three prime untranslated region transcript variants in cancer cell models

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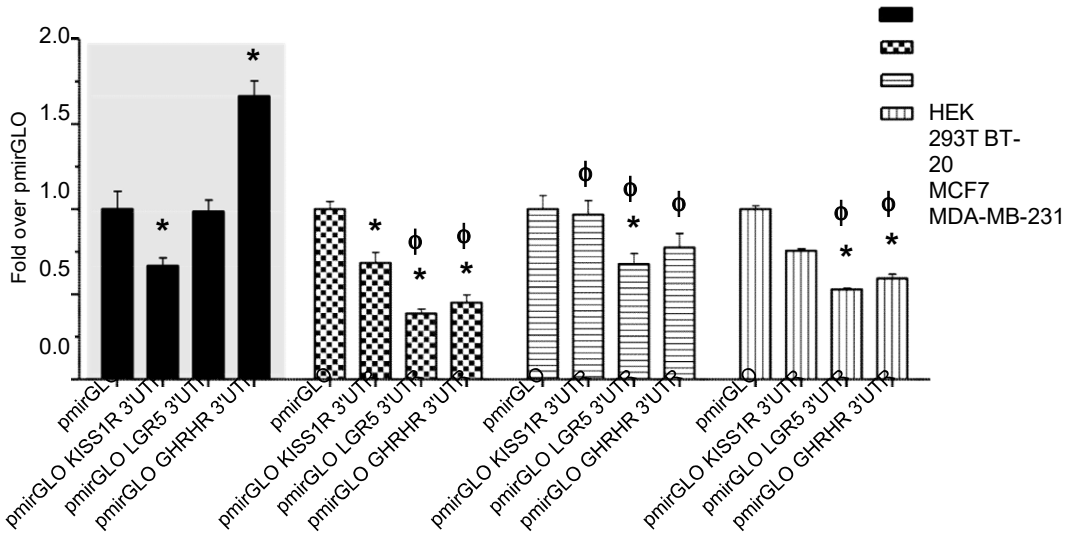
Baseline-corrected luciferase expression of KISS1R, LGR5 and GHRHR 3'UTRs in breast cancer cell lines 48 hours post transfection



Introduction: G protein-coupled receptors (GPCRs) are integral membrane proteins, comprising one of the largestgene families. GPCR signalling affects almost all human physiological systems, and as such the expression of the receptors and their cognate ligands are subjected to strict regulation. Dysregulated GPCR expression has been associated with an array of pathological conditions, including cancer. While most research focuses on the regulation of GPCR expression at a transcriptional level, the role of the mRNA 3' untranslated region (UTR) in post-transcriptional regulation of GPCR expression remains largely unexplored. Accordingly, this study set out to determine whether the expression of GPCRs implicated in breast cancer (KISS1R, LGR5 and GHRHR) are regulated post-transcriptionally by their 3' UTRs.

Methodology: The 3' UTRs of the three GPCRs of interest were cloned into pmirGLO vector downstream of a firefly luciferase open-reading frame (ORF). These constructs were then transfected into the four cell lines and luciferase activity was measured using a dual luciferase assay to quantify the effects of the 3' UTRs on luciferase translation. The pmirGLO vector is a dual cassette construct as it also contains a *Renilla* luciferase ORF to permit normalisation of the firefly luciferase signal, to control for transfection efficiency. pmirGLO containing no 3' UTR downstream ofthe firefly luciferase ORF was used as a negative control (empty vector).

Results: In HEK-293T cells The KISS1R 3' UTR significantly decreased luciferase expression in HEK-293T and BT-20 cells, when compared to empty vector. In contrast, GHRHR 3' UTR significantly increased luciferase expression in HEK-293T cells but showed a significant decrease in luciferase expression in BT-20 and MDA-MB-231 cells. Meanwhile, the LGR5 3' UTR shows a significant decrease in all the breast cancer cell lines when compared to the empty vector control in each cell line. Statistical analysis comparing the effects on luciferase expression between HEK-293T (non-cancer) and breast cancer cell lines demonstrates a significant decrease in luciferase expression mediated by the LGR5 3' UTR and GHRHR 3' UTR in all the breast cancer cell lines. In contrast, luciferase expression increases significantly in MCF7 cells compared to HEK-293T cells in the presence of KISS1R 3' UTR.

Discussion and Conclusion: We demonstrate for the first time that cancer associated GPCRs (KISS1R, LGR5 and GHRHR) may be differentially expressed in breast cancer cell backgrounds compared to a non-cancer cell background and that this regulation may in part be due to 3' UTR-mediated translational regulation.

Keywords: Three prime untranslated region (3' UTR), translational regulation, post-transcriptional regulation, Kisspeptin receptor (KISS1R), leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5), growth hormone-releasing hormone receptor (GHRHR)