EP810: Monocyte ghrelin gene-derived peptides – culprits in fat accumulation and obesity?

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INTRODUCTION

It is now appreciated that peptide hormones encoded by the ghrelin gene, *GHRL*, have roles in many biological systems and cell types (1). In particular, the hormone ghrelin is a therapeutic target and clinical marker for a range of pathologies, including diet-induced and genetic obesity.

Here, we interrogated publicly-available transcriptome (RNA-seq) data to investigate the expression of *GHRL* in a battery of cells and tissues.

METHODS

FASTQ Illumina transcriptome data (RNA-seq; each with a minimum of three biological replicates), representing 35 human adult somatic tissue or cell types, were obtained from the HPA (2) and ENCODE (3) consortia.

To link monocyte *GHRL* more directly to functional outcomes, we next sought to compare its expression before bariatric surgery and 12 weeks postoperatively in 23 women, interrogating an RNA-seq data set with two technical replicates for each time point (NCBI GEO accession number; 4). In accordance with the Fort Lauderdale agreement on fair use of community resource data (see http://www.genome.gov/10506537), transcriptomes were only examined for *GHRL*, its acylating enzyme GOAT, (*MBOAT4*), and its cognate receptor (*GHSR*).

All RNA-seq data were from paired-end Illumina libraries. FASTQ files were aligned to the human genome, UCSC build hg19 (using the spliced-read mapper TopHat, v2.0.9) (5), and reference gene annotations to guide the alignment. Raw gene counts were computed from TopHat-generated BAM files using featureCounts v1.4.5-p1 (6) and normalised using the quantile method available in the R package 'preprocessCore'. A paired Student's t-test was used for two-group comparisons, with $P \leq 0.05$ considered significant.

RESULTS

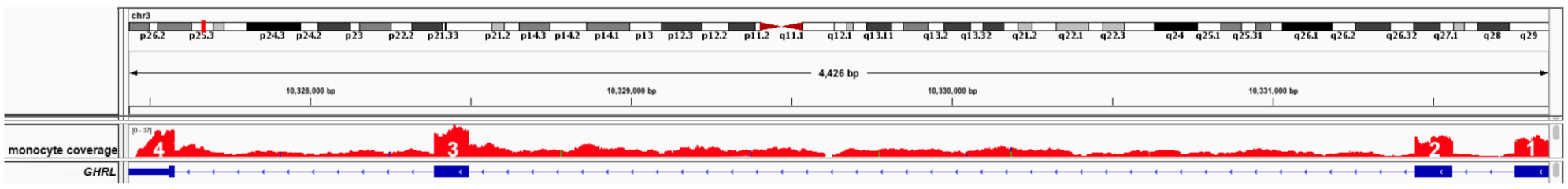


Figure 1 (above). Integrative Genomics Viewer (IGV) visualisation of the *GHRL* locus in monocytes validates expression of all canonical preproghrelin-coding exons (1-4).

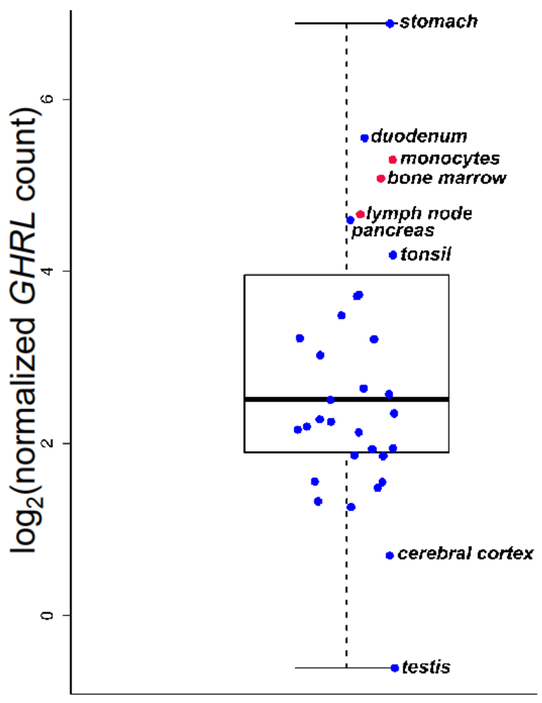


Figure 2 (left). Box plot of *GHRL* expression in 35 cells and tissues, from the Human Protein Atlas (HPA) and ENCODE, reveals high expression in **monocytes and associated tissues**.

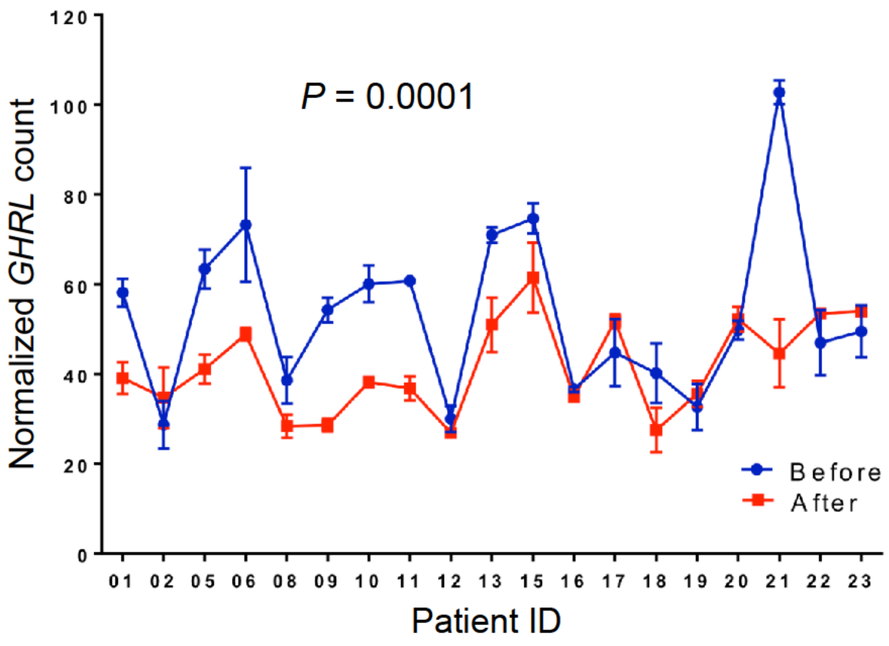
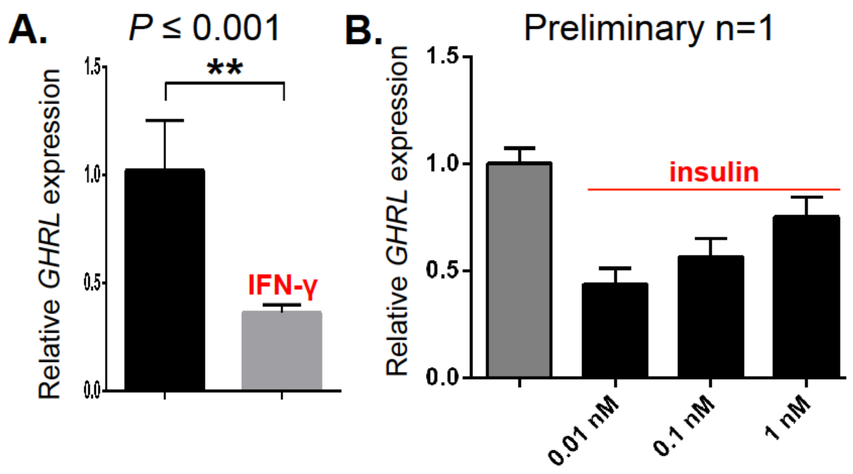


Figure 3. Monocyte *GHRL* expression is reduced 3 months after bariatric surgery (After) compared to levels pre-surgery (Before). Mean ± s.e.m. *P* value: paired Student's t-test.

- ✓ High *GHRL* expression in monocytes and associated tissues
- ✓ Bariatric surgery reduces monocyte *GHRL* expression 12 weeks postoperatively
- ✓ The proinflammatory cytokine interferon-γ (IFN-γ) and insulin are dysregulated in obesity (4,7-8)
- Q: Is reduced postoperative monocyte *GHRL* expression due to the altered levels or signalling of circulating molecules and an improved inflammatory profile?



Although the THP-1 monocytic leukemia transcriptome is quite distinct to primary monocytes and monocytes in *vivo* (9), we show that **IFN-γ and insulin can modulate *GHRL* expression in a monocyte-derived cell line**.

Figure 4. qRT-PCR demonstrating *GHRL* expression in the human THP-1 monocytic leukaemia cell line treated with **A.** 50 ng/ml interferon-γ (IFN-γ) for 24 h (n=5 in each group), compared to vehicle-treated controls. **B.** THP-1 monocytic leukaemia cell line treated with 0.01-1.0 nM insulin. Typical insulin levels are 0.09 nM before surgery and 0.03 nM after bariatric surgery (8). Mean ± s.e.m. ** *P* value ≤ 0.01 Mann–Whitney U test.

CONCLUSIONS

- While it is well-established that ghrelin plays a role in appetite regulation and energy balance, the function of *GHRL* in immune cells has remained enigmatic. Here, we present data that further supports its role in **cross-talk between the endocrine and immune systems**.
- We hypothesise that monocyte *GHRL*-derived hormones are critical mediators of the brain-gut axis (11) and monocyte-adipocyte cross-talk (12).
- Future longitudinal studies are needed to firmly establish a role for monocyte *GHRL*-derived peptides in successful bariatric surgery and obesity-associated pathologies, such as Prader-Willi syndrome and metabolic syndrome, in general.

References:1) J Endocrinol 2014; 220(1): R1-24. 2)Science 2015; 347(6220): 1260419. 3) Nature 2012; 489(7414): 57-74. 4) PLoS One 2015; 10(5): e0125718. 5) Genome biology 2013; 14(4): R36. 6) Bioinformatics 2014; 30(7): 923-30. 7) Arterioscler Thromb Vasc Biol 2011; 31(9): 2063-9. 8) Diabetes 2013; 62(8): 2747-51. 9) J Atheroscler Thromb 2004; 11(2): 88-97. 10) The Journal of endocrinology 2015; 226(1): 81-92. 11) Cell Metab 2014; 19(5): 821-35

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