Abundant ghrelin gene expression by monocytes: Putative implications for fat accumulation and obesity

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A R T I C L E   I N F O

Article history:
Received 4 October 2016
Accepted 30 December 2016

Keywords:
Ghrelin
Monocytes
Obesity
Bariatric surgery
Gene expression
Adipose tissue

A B S T R A C T

Hormones encoded by the ghrelin gene, GHRL, regulate many body systems. Here, we show that GHRL is abundant in monocytes. Analysis of women subjected to bariatric surgery revealed a significant post-operative reduction of GHRL in monocytes. We hypothesise that GHRL mediates monocyte-adipocyte cross-talk in health and obesity.

1. Introduction

Ghrelin is a pleiotropic hormone with roles in appetite stimulation and energy balance (Gahete et al., 2014a). The ghrelin gene, GHRL, is predominately expressed in the stomach, and transcribed at lower levels in multiple cell types (Gahete et al., 2014a). Our laboratory has a long-standing interest in characterising the structure, expression and function of peptide hormones derived from GHRL (Seim et al., 2013, 2016a). In this study, we initially assessed the expression of GHRL in 35 cell or tissue types by interrogating publicly available RNA-seq data.

2. Material and methods

2.1. Gene expression data processing

RNA-sequencing data, from 35 human adult somatic tissue or cell types (each with a minimum of three biological replicates), were obtained from the Human Protein Atlas (Uhlen et al., 2015) and ENCODE (Dunham et al., 2012) consortia and interrogated for ghrelin gene (GHRL) expression. For sample details see Seim et al., 2016b. To further investigate GHRL expression in monocytes, we examined an RNA-seq data set from 19 women before and three months after bariatric surgery, each with two technical replicates (NCBI GEO: GSE66306). FASTQ files were aligned to the human genome (hg19) using TopHat (v2.0.9) (Kim et al., 2013). Gene counts were computed by featureCounts v1.4.5-p1 (Liao et al., 2014) and normalised using the quantile method (R package ‘preprocessCore’). A paired Student’s t-test was used for two-group comparisons, with P ≤ 0.05 considered significant.

2.2. GHRL expression in THP-1 cells treated with interferon-γ

The human acute monocytic leukaemia cell line THP-1 (ATCC TIB-202) was treated with interferon-γ (IFN-γ) (50 ng/ml; R&D Systems) or vehicle (PBS) for 24 h. RNA was extracted (High Pure RNA Isolation Kit, Roche), cDNA generated (iScript; Bio-Rad), and qRT-PCR (2X SYBR green) performed using an AB7500 Fast thermal cycler (Applied Biosystems). GHRL qRT-PCR was performed (assay cat. no. QTO0041377; QIAGEN) and normalised to the housekeeping
gene TATA box protein (TBP) (5'-TTCGCTCGGCTGGCCCATAG-3' and 5'-TCTCCAGCACACTCTTCTCAGCAA-3'). The relative expression of GHRL was calculated using the comparative 2^{ΔΔCt} method. IFN-γ treated and control groups were compared using the Mann-Whitney U test (P ≤ 0.05 considered significant).

3. Results and discussion

Surprisingly, GHRL expression was relatively abundant in monocytes and lymphoid tissues, such as bone marrow and lymph nodes (Fig. 1A). An assessment of exon coverage in monocytes confirmed expression of canonical preproghrelin coding exons 1 to 4 (Fig. 1B), as described for peripheral blood mononuclear cells (Dixit et al., 2004; Gahete et al., 2014b).

As the function of GHRL in immune cells and its relationship with disease has hitherto remained enigmatic, we examined transcriptomes of 19 women before and three months after bariatric surgery (Poitou et al., 2015). Our analysis revealed significantly reduced GHRL expression in monocytes after surgery (P = 0.0001) (Fig. 1C and Table 1), but no difference in adipocytes from the same cohort (data not shown). Serum and enteroenodocrine ghrelin production are reduced in most patients following bariatric procedures (Meek et al., 2016), and we now demonstrate that monocyte GHRL expression is also modulated.

Reduced GHRL expression in monocytes postoperatively may result from altered expression or signalling of GHRL-regulating molecules (e.g. leptin and cytokines), or from improved glucose homeostasis. In blood and tissues, the pro-inflammatory cytokine interferon-γ (IFN-γ) is elevated in obesity and reduced following bariatric surgery (Zhang et al., 2011; Monteiro-Sepulveda et al., 2015), and there is evidence that IFN-γ may regulate GHRL expression. GHRL expression was significantly decreased in human

Table 1

| Patient ID | Type of surgery | Normalised GHRL gene count | Direction of expression change |
|------------|-----------------|---------------------------|------------------------------|
|            | Before Mean    | Before S.E.M. | After Mean  | After S.E.M. |                 |
| PM01       | AGB            | 58.2          | 3.1         | 39.1         | 3.6          |
| PM02       | RYGB           | 28.7          | 5.3         | 34.7         | 6.8          |
| PM05       | RYGB           | 63.4          | 4.3         | 41.1         | 3.2          |
| PM06       | RYGB           | 73.3          | 12.7        | 48.9         | 1.4          |
| PM08       | RYGB           | 38.6          | 5.2         | 28.4         | 1.4          |
| PM09       | AGB            | 54.3          | 2.6         | 28.6         | 1.4          |
| PM10       | RYGB           | 60.1          | 4.1         | 38.2         | 1.2          |
| PM11       | RYGB           | 69.8          | 0.0         | 36.8         | 2.7          |
| PM12       | RYGB           | 30.0          | 2.9         | 26.9         | 0.7          |
| PM13       | AGB            | 71.0          | 1.8         | 51.0         | 6.1          |
| PM15       | RYGB           | 74.7          | 3.3         | 61.5         | 7.8          |
| PM16       | AGB            | 36.6          | 0.6         | 34.8         | 0.4          |
| PM17       | RYGB           | 44.8          | 7.5         | 51.8         | 1.5          |
| PM18       | AGB            | 40.2          | 6.7         | 27.5         | 5.0          |
| PM19       | RYGB           | 32.7          | 5.1         | 35.6         | 2.9          |
| PM20       | AGB            | 49.8          | 2.1         | 52.2         | 2.8          |
| PM21       | RYGB           | 102.8         | 2.6         | 44.6         | 7.6          |
| PM22       | AGB            | 47.0          | 7.3         | 53.5         | 0.3          |
| PM23       | AGB            | 49.5          | 5.8         | 54.0         | 1.0          |
THP-1 monocytic leukaemia cells treated with IFN-γ (Fig. 1D; \( P = 0.0079 \)), suggesting that IFN-γ signalling can potentially modulate GHRL expression in this monocyte-derived cell line. Indeed, the bariatric surgery cohort examined here showed evidence of a distinct IFN-γ signalling pathway in adipose tissue and monocytes postoperatively (Poitou et al., 2015). IFN-γ represses gastric GHRL expression in non-obese mice infected with Helicobacter pylori (Strickertsson et al., 2011) and IFN-γ regulation has also been associated with dietary interventions in rodents (Lee et al., 2006; Poitou et al., 2015). Taken together, we speculate that weight reduction modulates the expression of GHRL via an altered IFN-γ signalling pathway.

What are the consequences of reduced monocyte GHRL expression after bariatric surgery? Obesity induces inflammation in adipose tissue, and metabolic improvements after bariatric surgery are mediated by the reversal of endocrine and immune responses associated with ‘pathogenic’ adipose tissue. A very recent study demonstrated that inflamed adipose tissue directly stimulates aberrant monocyte production in the bone marrow (monocytosis) to further exacerbate obesity-associated disease processes (Nagareddy et al., 2014). The GHRL derived peptide hormones ghrelin and obestatin regulate adiposity and may promote associated inflammation in adipose tissue (Tsubone et al., 2005; Rodriguez et al., 2009; Gurriaran-Rodriguez et al., 2011). We hypothesise that obesity-associated monocytosis and inflammation, coupled with the action of monocyte-derived paracrine GHRL-derived hormones on adipocytes, regulates metabolism and adipogenesis. Conversely, reduced monocyte GHRL expression following bariatric surgery may improve adipose tissue function.

Monocyte GHRL expression was reduced in ~60% of subjects 12 weeks postoperatively, while expression was not altered in the remainder (Table 1). Importantly, the patients were still markedly obese (Poitou et al., 2015). It would be of interest in future studies to examine whether monocyte GHRL expression is reduced for longer than three months after surgery and correlates with successful longer-term bariatric surgery outcomes. In addition, it would be useful to examine the expression of monocyte GHRL in other obesity-associated pathologies, such as Prader-Willi syndrome (where patients exhibit hyperghrelinaemia and hyperphagia from an early age) and metabolic syndrome in general (a risk factor for cancer). Our study demonstrates the power of mining public genome-wide gene expression data and provides further impetus into the study of cross-talk between the endocrine and immune systems and the ghrelin axis.

**Conflict of interest statement**

The authors declare no conflict of interest.

**Authors’ contributions**

IS conceived the study, performed computational biology analyses, and wrote the first draft of the manuscript. GC and ETS performed wet-laboratory experiments, under supervision of PLJ. IS, PLJ and LKC analysed and interpreted results, and edited the manuscript. All authors read and approved the final version of the manuscript.

**Acknowledgements**

The THP-1 cell line was a gift from Dr. Rebecca Pelekanoos (University of Queensland Centre for Clinical Research).

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