Presence and functional actions of In1-ghrelin splicing variant reveals a potentially relevant pathophysiological role in human pituitary adenomas

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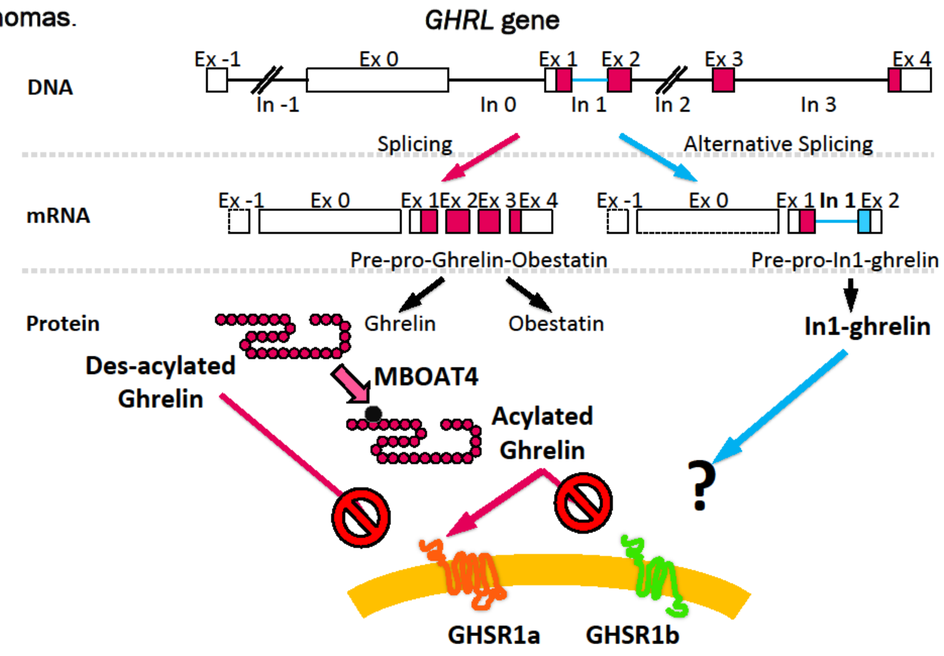
Introduction

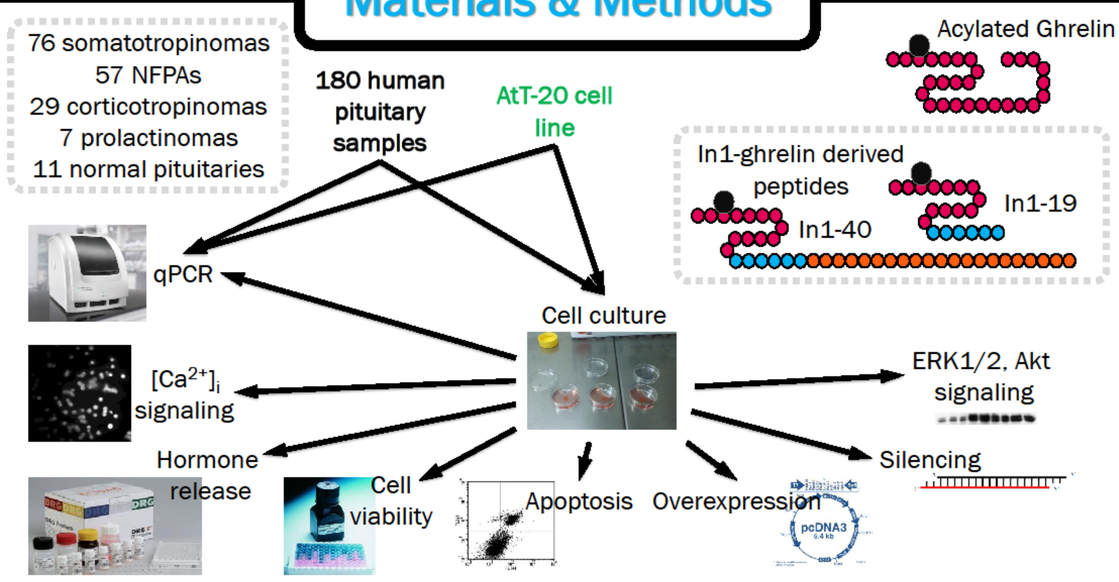
Pituitary adenomas comprise a heterogeneous group of tumors causing serious comorbidities, which would benefit from identification of novel, common molecular/cellular biomarkers and therapeutic targets. The **ghrelin system** encompass a complex molecular family with multiple functions, and some of its components have been linked to development of various endocrine-related cancers. In this work, we aim to better delineate the patho-physiological significance of the ghrelin regulatory system in pituitary tumors, by pursuing two specific objectives:

1) To analyze the presence of key components of the ghrelin system in pituitary tumors: **native-ghrelin**, the recently discovered splicing variant **In1-ghrelin**, ghrelin receptors **GHS-R1a** (full-length) and **GHS-R1b** (truncated variant), and **MBOAT4** (GOAT), the enzyme responsible for ghrelin acylation

2) To compare the direct effects of **native-ghrelin** and **In1-ghrelin** variant administration on selected **functional parameters** in cell cultures derived from the main types of **pituitary adenomas**.



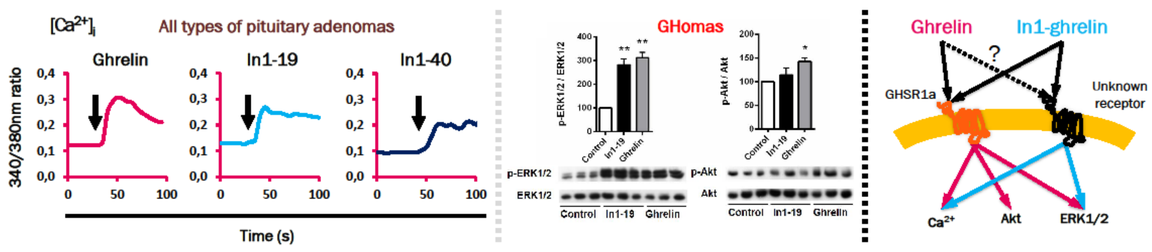
Materials & Methods



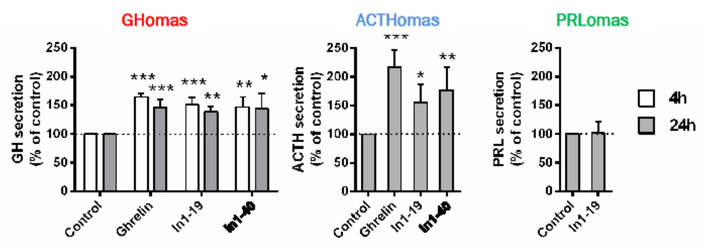
Functional assays

In1-ghrelin derived peptides and native ghrelin induced differential intracellular signaling activation in pituitary adenoma cells

Intracellular signaling

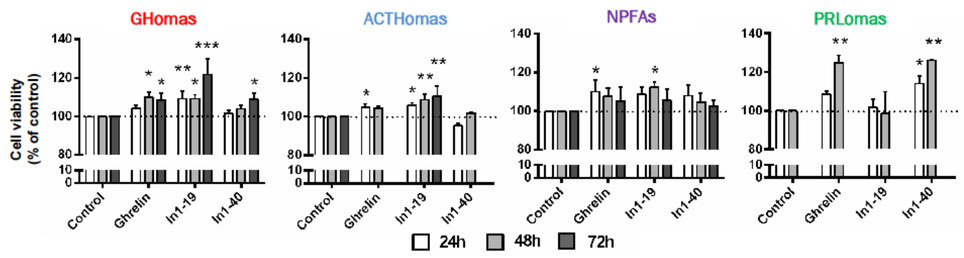


Hormone secretion

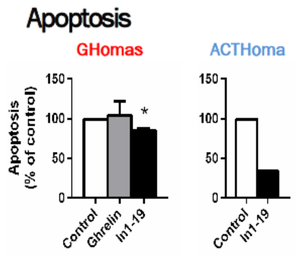


Ghrelin and In1-ghrelin peptides similarly stimulated hormone release in **GH-** and **ACTH-** secreting adenomas, whereas, In1-19 was not able to alter PRL secretion from **prolactinoma** cell cultures.

Cell viability



Apoptosis

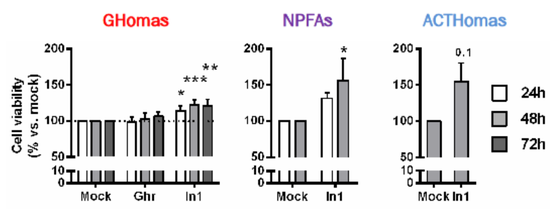


Administration of acylated native ghrelin and In1-ghrelin peptides **increased cell viability** *in vitro* in all pituitary adenoma subtypes.

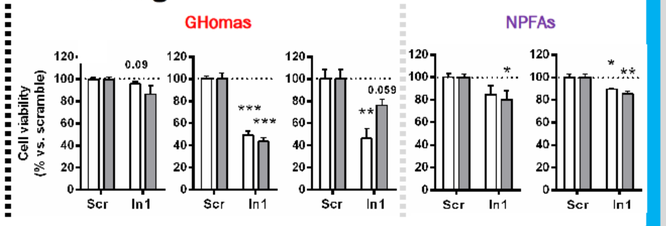
The administration of In1-19 **reduced apoptosis** *in vitro* in GHomas, being this effect similar in one ACTHoma analyzed.

In1-ghrelin **overexpression increased cell viability**, while the use of a specific **siRNA** against In1-ghrelin **reduced cell viability** in GHomas and NFPAs.

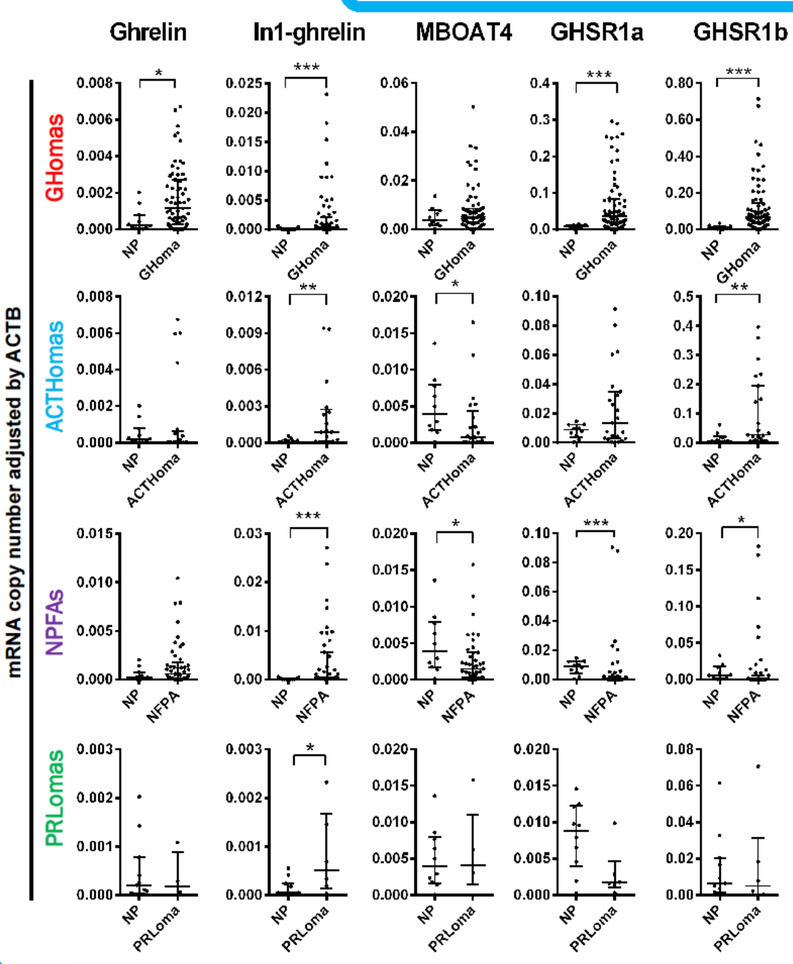
Overexpression



Silencing



Expression profile



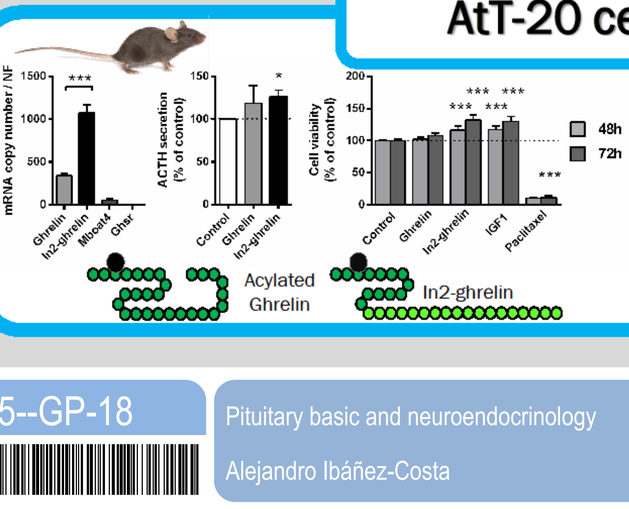
All components of the ghrelin system examined: ghrelin, In1-ghrelin variant, GHSR1a, GHSR1b and MBOAT4 enzyme, were expressed in normal pituitaries and pituitary adenomas.

We observed that ghrelin system was altered in pituitary adenomas compared to normal pituitary.

In1-ghrelin expression was **consistently elevated** in all pituitary adenoma subtypes.

Additionally, the expression of In1-ghrelin was **correlated** with that of **MBOAT4** in pituitary adenomas, and not in normal pituitaries.

AtT-20 cell line



In mouse corticotropinoma cell line, AtT-20, we also observed a significant overexpresion of **In2-ghrelin**, mouse In1-ghrelin counterpart. Ghrelin did not significantly alter basal ACTH release or cell viability, maybe due to the lack of Ghsr expression, while treatment with **In2-ghrelin** peptide significantly **increased** basal **ACTH release** and **cell viability**.

Conclusions

Altogether, our results indicate that ghrelin system components are present and markedly altered in human pituitary tumors, where **In1-ghrelin** variant, particularly, **could play a relevant functional role** in the **regulation** of **adenoma pathology**, which pave the way for using In1-ghrelin variant as a new tool to explore novel diagnostic/prognostic biomarkers and/or therapeutic targets in these human tumors.

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