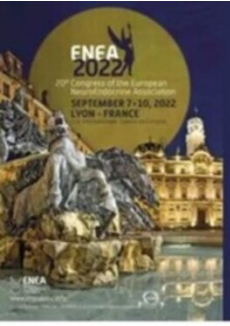


Somatostatin and ghrelin systems characterization and therapeutic potential in liver diseases

N. Herman-Sánchez[1], A. García-Estrada[1], JL. López-Cánovas[1], P. Sáez-Martínez[1], J. Córdoba-Chacón[2], A. Sarmento-Cabral[1], M. Rodríguez-Perálvarez[1], RM. Luque[1], MD. Gahete[1]

1 Maimónides Institute of Biomedical Research of Córdoba (IMIBIC), Córdoba, SPAIN, 2 Department of Medicine, Division of Endocrinology, Diabetes and Metabolism, University of Illinois at Chicago, Illinois, UNITED STATES

INTRODUCTION

The components of the somatostatin (SST), cortistatin (CORT), neuronostatin (NST) and ghrelin systems are useful biomarkers in different endocrine and neuroendocrine cancers and their synthetic analogues are valuable tools for their clinical management. However, the role of SST, CORT, NST and ghrelin signalling in liver diseases is poorly known.

We characterised the presence of the components of the SST/CORT/NST and ghrelin systems and evaluated their clinical potential in metabolic dysfunction-associated fatty liver disease (MAFLD) and hepatocellular carcinoma (HCC)

METHODS

RETROSPECTIVE-1

N=93 (tumor vs. non-tumor adjacent tissues)

RETROSPECTIVE-2

N=58 (tumor vs. non-tumor adjacent tissues)

N=39 (cirrhosis)

N=5 (healthy livers)

LIVER CANCER CELL LINES

HepG2 | Hep3B | SNU-387

MOUSE DIETS

CDAHFD | HFHCHFD | MCD

RNA extraction | Retrotranscription | qPCR (Fluidgm Array)

FLUIDIGM

SST | SSTR3 | SSTR5 | Ghrelin | GPR107 | SSTR1 | SSTR4 | SSTR5TMD3 | IN1-Ghrelin | In2-Ghrelin | SSTR2 | CORT | SSTR5TMD4 | GOAT | MRGPRX2

IN SILICO COHORTS

LIHC (N= 369 tumor vs. N=50 healthy)

Mas (N= 38 tumor vs. N=19 healthy)

Wurmbach (N= 35 tumor vs. N=10 healthy)

Zhou (N= 165 tumor vs. Non-tumor adjacent tissue)

Roessler 2 (N= 225 tejido tumoral vs. N=220 tejido sano)

Chen (N= 106 tumor vs. N=76 healthy)

Roessler (N= 22 tumor vs. N=21 healthy)

Guichard (N= 99 tumor vs. N=86 healthy)

Guichard 2 (N= 26 tumor vs. N=26 healthy)

Llovet (N= 6 healthy vs. N=8 cihrrosis vs. N=74 NASH vs. N=29 Adjacent vs. N=52 tumor)

Allard (N= 24 healthy vs. N=20 MAFLD vs. N=19 NASH)

PROLIFERATION ASSAYS

HepG2 | Hep3B | SNU-387 | Primary liver culture

SST | NST | CORT | LAN | PASIR | OCT

RESULTS

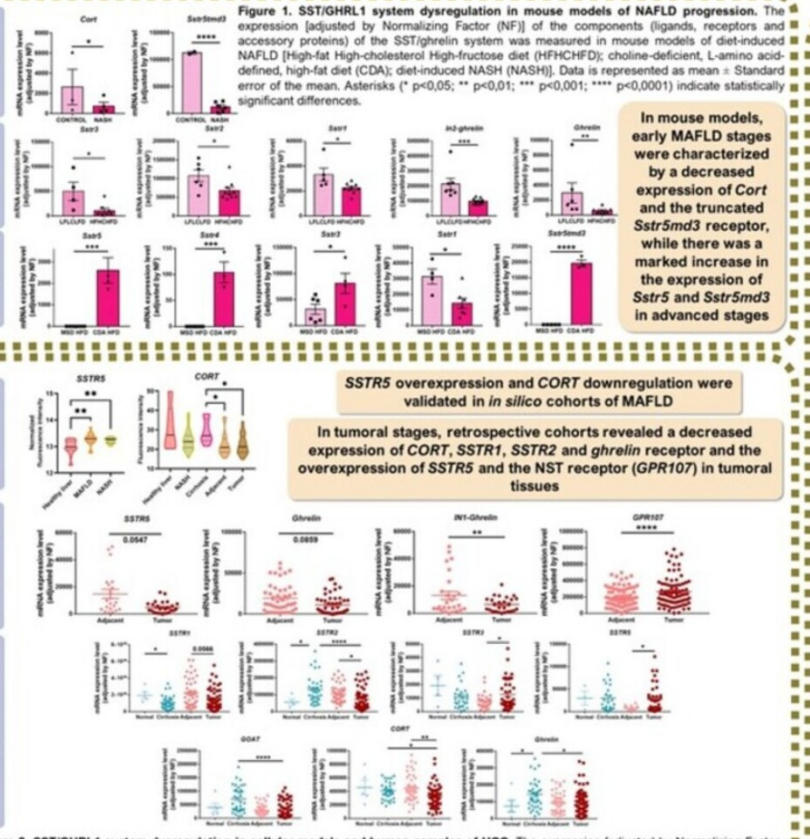


Figure 1. SST/GHRL1 system dysregulation in mouse models of NAFLD progression. The expression [adjusted by Normalizing Factor (NF)] of the components (ligands, receptors and accessory proteins) of the SST/ghrelin system was measured in mouse models of diet-induced NAFLD [High-fat High-cholesterol High-fructose diet (HFHCHFD); choline-deficient, L-amino acid-defined, high-fat diet (CDA); diet-induced NASH (NASH)]. Data is represented as mean ± Standard error of the mean. Asterisks (* p<0,05; ** p<0,01; *** p<0,001; **** p<0,0001) indicate statistically significant differences.

In mouse models, early MAFLD stages were characterized by a decreased expression of *Cort* and the truncated *Sstr5md3* receptor, while there was a marked increase in the expression of *Sstr5* and *Sstr5md3* in advanced stages

***SSTR5* overexpression and *CORT* downregulation were validated in *in silico* cohorts of MAFLD**

In tumoral stages, retrospective cohorts revealed a decreased expression of *CORT*, *SSTR1*, *SSTR2* and *ghrelin* receptor and the overexpression of *SSTR5* and the NST receptor (*GPR107*) in tumoral tissues

Figure 2. SST/GHRL1 system dysregulation in cellular models and human samples of HCC. The expression [adjusted by Normalizing Factor (NF)] of the components (ligands, receptors and accessory proteins) of the SST/ghrelin system was measured in human *in silico* cohorts of NAFLD and human samples from 2 HCC cohorts [Retrospective 1 (R1) (n=102 patients) (adjacent vs. tumoral tissue); Retrospective 2 (R2) (n=93 patients) (adjacent vs. tumoral tissue; cirrhosis; healthy)]. Data is represented as mean ± Standard error of the mean. Asterisks (* p<0,05; ** p<0,01; *** p<0,001; **** p<0,0001) indicate statistically significant differences.

In vitro assays revealed a decreased proliferation after treatment with SST, CORT, NST and the synthetic analogues

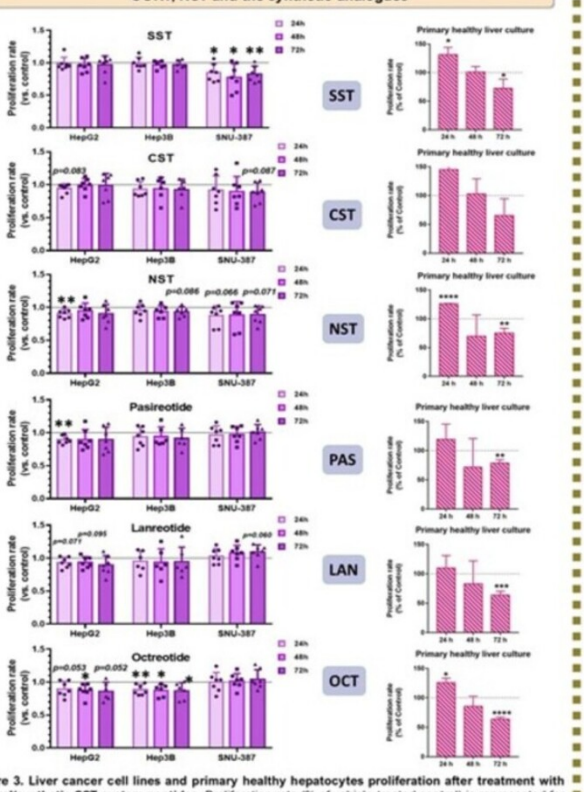
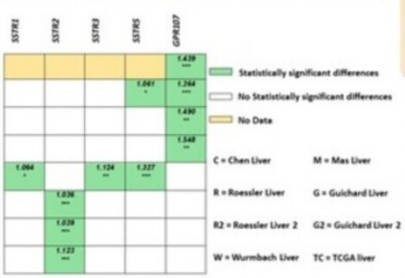
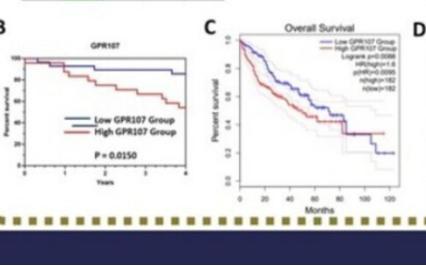


Figure 3. Liver cancer cell lines and primary healthy hepatocytes proliferation after treatment with natural/synthetic SST system peptides. Proliferation rate (% of vehicle-treated control) is represented for liver cancer cell lines (HepG2, Hep3B and SNU-387) and primary healthy liver hepatocytes after treatment with natural [Somatostatin (SST), Cortistatin (CORT), Neuronostatin (NST)] and synthetic [Pasireotide (PAS), Octreotide (OCT), Lanreotide (LAN)] SST system peptides. Data is represented as mean ± Standard error of the mean. Asterisks (* p<0,05; ** p<0,01; *** p<0,001; **** p<0,0001) indicate statistically significant differences.





***GPR107* overexpression was associated with key aggressiveness parameters (survival, recurrence, tumoral diameter, etc.) in the retrospective and *in silico* cohorts**

Figure 4. GPR107 dysregulation and association to aggressiveness in *in silico* and Retrospective-2 cohorts of HCC. (A) SST/CORT/NST receptors expression (mRNA) in *in silico* cohorts of HCC from Oncomine and The Cancer Genome Atlas (TCGA). Fold Change of statistically significant differences is represented. **(B)** Survival curve [high *GPR107* expression (Red, n=29) vs. low *GPR107* expression, (Blue, n=29)] in Retrospective-2 cohort. **(C)** Survival curve [high *GPR107* expression (Red, n=182) vs. low *GPR107* expression, (Blue, n=182)] in TCGA cohort. **(D)** Survival curve [high *GPR107* expression (Red, n=82) vs. low *GPR107* expression, (Blue, n=82)] and clinical/molecular correlations of *GPR107* expression (protein) in Zhou Cohort (CPTAC). Asterisks (* p<0,05; ** p<0,01; *** p<0,001; **** p<0,0001) indicate statistically significant differences.

CONCLUSIONS

This study demonstrates an ample alteration of SST/CORT/NST and ghrelin systems in liver pathologies, and suggests the prognostic and therapeutic potential of certain components of these hormonal systems (i.e. GPR107) and of SST-analogues for their clinical management.

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nataliahermansan@gmail.com