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Unraveling the RNA landscape of small intestine neuroendocrine neoplasms applying transcriptomic and spliceosomic perspectives

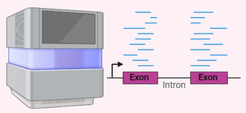
A Ibáñez-Costa[1], S Pedraza-Arevalo[1], MT Moreno-Montilla[1], V García-Vioque[1], J Hernando[2], A García[3], A García-Álvarez[2], S Landolfi[4], L Blanco[5], A Casteras[6], A Vivancos[6], J Capdevila[2], JP Castaño[1]

[1]Department of Cell Biology, Physiology and Immunology, University of Cordoba, Maimonides Biomedical Research Institute of Cordoba. Reina Sofia Hospital, Cordoba, Spain; [2]Medical Oncology, Vall d'Hebron University Hospital. Vall Hebron Institute of Oncology (VHIO), Barcelona, Spain; [3]Nuclear Medicine, Vall d'Hebron University Hospital, Barcelona, Spain; [4]Pathology, Vall d'Hebron University Hospital, Barcelona, Spain; [5]General Surgery, Vall d'Hebron University Hospital, Barcelona, Spain; [6]Endocrinology, Vall d'Hebron University Hospital, Barcelona, Spain

Background

The study of small **intestine neuroendocrine neoplasms** (**siNENs**) represents a challenge due to their rarity, tissue availability, and heterogeneity. Recent studies identified different transcriptomic molecular subtypes, but there are still major gaps in understanding the mechanisms driving **siNEN** progression. There is growing evidence that the **splicing process is altered in cancer**. Splicing is the mechanism responsible for RNA maturation through intron excision and exon junction. We have previously shown that splicing is dysregulation in various types of NENs, including pancreatic and lung NENs, identifying both aberrantly spliced variants and altered components of the splicing machinery, which were associated with and clinical parameters and increased tumor aggressiveness features. In contrast, **the role of splicing in siNENs and its putative relation to tumor biology is still largely unexplored**. Therefore, the aim of this study is to evaluate the spliceosomic landscape, —splicing factors and variants—, in a set of 44 **siNENs** to identify putative new biomarkers related to clinical features.

Methods



44 siNEN FFPE samples
- **27 long-term survivors**
- **17 short-term survivors**

Collect RNA Sample → Sequencing library → Sequence reads

Sequence reads → align reads to transcriptome — **STAR**

align reads to transcriptome → quantify gene expression — **htseq-count**

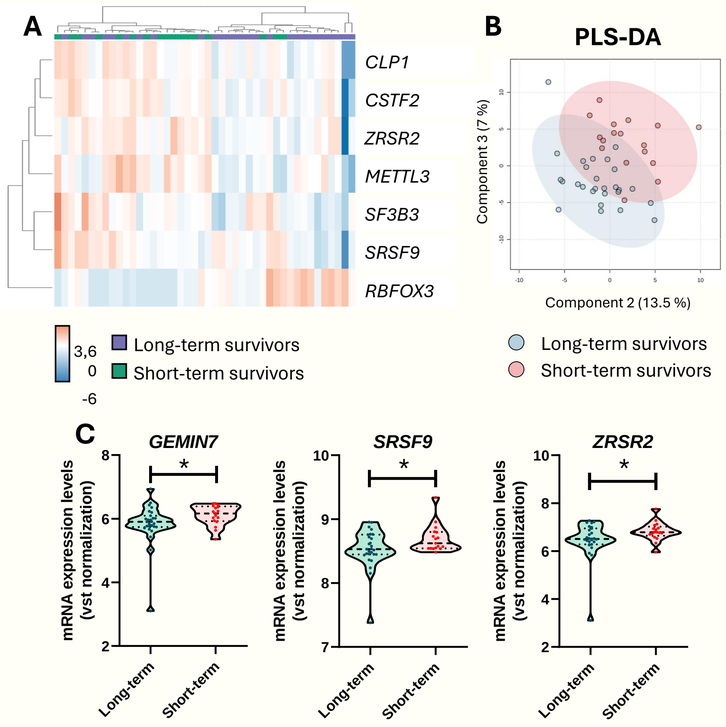
align reads to transcriptome → quantify alternative splicing — **rMATS**

quantify gene expression → identify differentially expressed genes — **DESeq2**

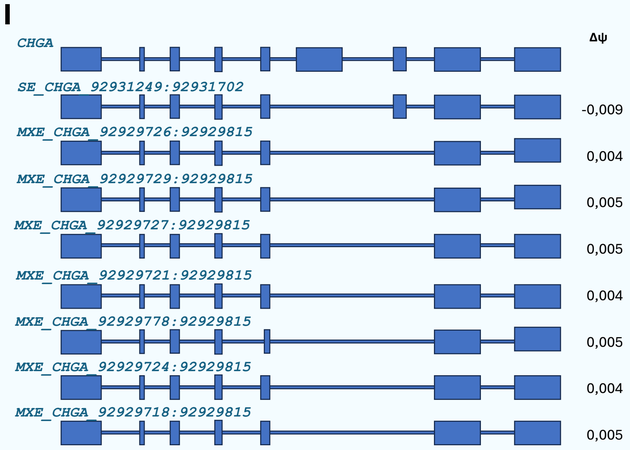
quantify alternative splicing → identify splicing events — **rMATS**

Results

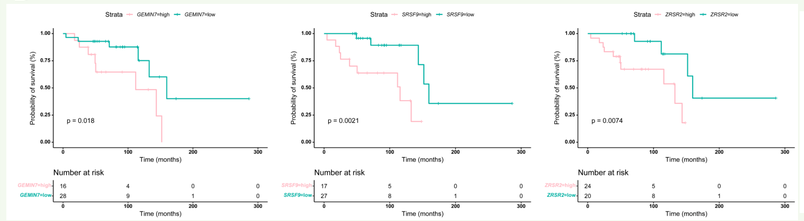
We quantified 60,607 transcripts in the 44 siNENs. Unsupervised analysis of the splicing machinery did not discriminate distinct subtypes as shown by heatmaps (**A**) or regression analyses (**B**). However, we identified 3 genes whose expression differed between short- and long-term survivors (**C**).



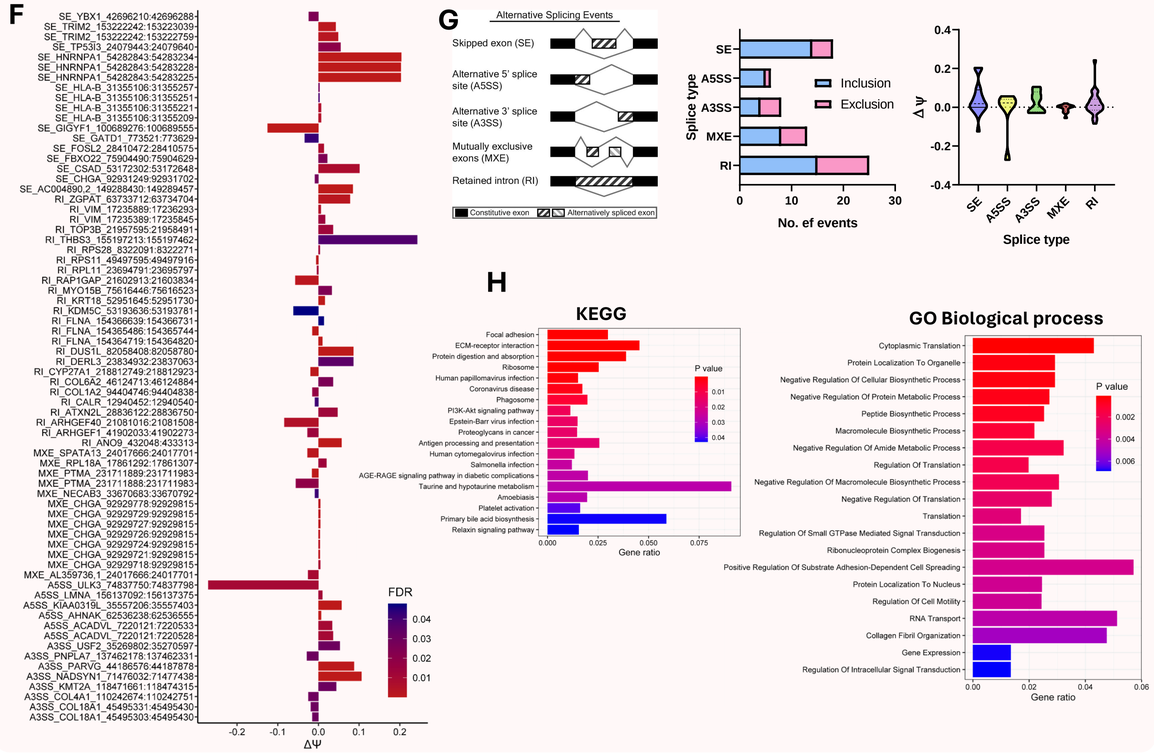
Interestingly, we observed notable number of differentially spliced Chromogranin A (*CHGA*, **I**) variants among patients according to their survival, some of them were not previously described.



GEMIN7, *SRSRF9* and *ZRSR2* were associated to **patient survival**. These three splicing-related genes were differentially expressed between long-term and short-term survivors, and their high/low expression was directly associated with overall survival (**D**). Interestingly, the expression of two factors was also related to liver metastases (**E**).



Of the 434,286 splicing events detected, 70 were differentially spliced between patients with long and short survival (**F**). Of which, the majority were intronic retentions and skipped exons (**G**). Enrichment analyses demonstrated that the alternative splicing events affect genes belonging to key pathways such as translation, protein biosynthesis and focal adhesion (**H**).



Conclusions

Analysis of the splicing machinery and splicing variants enables the identification of altered genes and variants associated with specific clinical features, thus paving the way to enhance our understanding of siNEN heterogeneity and guiding future research to develop personalized targeted therapeutic strategies.

b12ibcoa@uco.es

@HYCLab