PROJECT

Supervisor:	Alessandro Sessa
Title:	**The role of microexon regulation in tumorigenesis**
Curriculum:	BAIO
Link to the personal page of the University or relevant hospital site website:	https://research.hsr.it/en/divisions/neuroscience/neuroepigenetics.html

Description of the Project (max 3,000 characters including spaces)

Background/gap of knowledge

Microexons, defined as exons between 3 and 27 nucleotides long, represent a distinct functional class of highly evolutionarily conserved exons (1-3) which require specialized machinery for alternative splicing (AS) (2-4). Specifically, the RNA-binding proteins SRRM3/4 regulate microexon inclusion through their "enhancer of microexons" (eMIC) protein domain (3,5,6). A recent study indicates that microexon inclusion is suppressed in tumor, possibly leading to tumor growth, while dose-dependent upregulation of SRRM4 inhibits proliferation (7).

We recently identified a SRRM3 isoform that includes the exon 15. This isoform produces a protein with an alternative C-term, resulting in the partial loss of the eMIC domain. Our preliminary data showed that, differently from the canonical SRRM3, the novel isoform has impaired capability to induce spontaneous Liquid-liquid phase separation (LLPS) (8), shows mislocalization, and mediates microexon exclusion.

Both SRRM3 and SRRM4 are expressed in human NB samples, where microexons showed moderate level of inclusion, with higher expression in low-risk patients (9). However, SRRM3 exon 15 inclusion correlates with worse prognosis. NB is a cancer that arises from differentiation arrest of the neural-crest-derived sympathoadrenal lineage, primarily affecting children (10). Current treatment involves intense chemoradiotherapies, thus novel therapeutic targets could not only improve the outcome, but could also reduce complications for patients.

Rationale and hypothesis

The novel SRRM3 isoform should function by counteracting the activities of the canonical SRRM3 and SRRM4. This regulation may involve direct competition for binding sites or modulation of

RNA/protein complex formation, leading to alterations in microexon inclusion profiles. We hypothesize that the novel SRRM3 isoform plays a critical role in the relatively poor microexon inclusion observed in tumors and thus contributing to malignancy.

Objectives and specific aims

The overall goal of this proposal is to advance the development of precision therapies for neuroblastoma through a deeper understanding of microexon inclusion and its regulation. Through multiple approaches, this proposal intends to reveal the precise mechanism of action of the novel SRRM3 isoform. It also proposes to generate reagents to specifically counteract novel SRRM3 isoform activity and promote antitumoral effects in experimental models of neuroblastoma. Aims:

1. Elucidate the microexon-associated SRRM3 complexes

2. Identify the specific targets of SRRM3 novel isoform

3. Define a novel therapeutic strategy for neuroblastoma

Expected outcomes

We expect to understand a previously overlooked regulatory mechanism of microexon inclusion, offering deeper insights into its potential role in tumorigenesis and therapeutic targeting. Our proposed therapeutic strategy aims to selectively modulate SRRM3 splicing to suppress the expression of this harmful isoform.

Skills that the student should acquire (max. 600 characters including spaces):

The student will perform molecular cloning, targeted mutagenesis, cell culture, evaluation of protein distribution in cells using confocal microscopy, evluation of microexon splicing by PCR analysis. Design and test of ASO candidates. In vivo transplant of cancer cells, evaluation of tumor growth. The student will also learn how to manage murine colonies and prepare them for experimental needs.

References (max. 15)

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2. Ustianenko D, Weyn-Vanhentenryck SM, Zhang C. Microexons: discovery, regulation, and function. Wiley Interdiscip Rev RNA. 2017

3. Irimia M, Weatheritt RJ, Ellis JD, Parikshak NN, Gonatopoulos-Pournatzis T, et al. A highly conserved program of neuronal microexons is misregulated in autistic brains. Cell. 2014

4. Gonatopoulos-Pournatzis T, Blencowe BJ. Microexons: at the nexus of nervous system development, behaviour and autism spectrum disorder. Curr Opin Genet Dev. 2020

5. Gonatopoulos-Pournatzis T, Wu M, Braunschweig U, Roth J, et al. Genome-wide CRISPR-Cas9 Interrogation of Splicing Networks Reveals a Mechanism for Recognition of Autism-Misregulated Neuronal Microexons. Mol Cell. 2018

6. Ciampi L, Mantica F, López-Blanch L, Permanyer J, Rodriguez-Marín C, et al. Specialization of the photoreceptor transcriptome by Srrm3-dependent microexons is required for outer segment maintenance and vision. PNAS. 2022

7. Head SA, Hernandez-Alias X, Yang JS, Ciampi L, Beltran-Sastre V, et al. Silencing of SRRM4 suppresses microexon inclusion and promotes tumor growth across cancers. PLoS Biol. 2021

8. Gomes E, Shorter J. The molecular language of membraneless organelles. J Biol Chem. 2019

9. SEQC/MAQC-III Consortium. A comprehensive assessment of RNA-seq accuracy, reproducibility and information content by the Sequencing Quality Control Consortium. Nat Biotech. 2014

10. De Bona A, Barbieri M, Rinaldi N, Esposito S. Neuroblastoma in Childhood: Biological Insights, Risk Stratification, and Advances in Multimodal Therapy. J Clin Med. 2026

11. Havens MA, Hastings ML. Splice-switching antisense oligonucleotides as therapeutic drugs. Nucleic Acids Res. 2016