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International PhD Program IC-PhD

PhD thesis project

2024 Call for application

Unravelling RNA G4 functions in alternative splicing events: underlying the role of G4 ligand stabilization

General information

Call	2024
Reference	2024-15-VERGA_MERGNY
Keyword(s)	RNA G-quadruplex; alternative splicing; G4 recognition; bioinformatics; high-grade glioma.

Director(s) and team

Thesis director(s)	Daniela Verga & Jean-Louis Mergny
Research team	Targeting of Nucleic Acids and Photolabelling Approaches
Research department	UMR9187 / U1196 - Chemistry and Modelling for the Biology of Cancer

Description of the PhD thesis project

Abstract

Alternative splicing (AS) occurs in the majority of human genes, and it is a vital mechanism for increasing complexity of gene expression during development or in response to cellular stimuli. AS landscape of high-grade glioma (HGG) determined by multi-omics analyses, showed how AS is strongly deregulated in cancer genes compared with normal brain and such alteration is associated with worse prognosis. RNA G-quadruplex (G4) location in the transcriptome and their actual formation in cells are important aspects for further exploring G4 functions and their significance in biology. Recently, bioinformatics studies have shown that G4 motifs are enriched near splicing junctions in mammals, and the importance of G4 in RNA is highlighted by a higher enrichment for the non-template strand. However, transcriptional approaches focusing on the use of G4 ligands able to target G4 RNA associated to cancer-related AS have been poorly explored. The aim of this project is to identify the impact produced by folding of G4 in AS events in cancer genes associated to HGG and explore the possibility to target G4 to modulate splicing for therapeutic purposes. Precisely, this interdisciplinary project involves the identification of RNA G4-prone sequences in proximity of splicing sites employing bioinformatics, biophysical and biochemical assays and the used of G4 ligands and RNA-seq experiments to identify RNA isoforms produced prior and after ligand treatment.

Background

Alternative splicing (AS) is essential to increase transcript diversity but has also the potential to affect cancer genes through removal or alteration of protein domains and post-transcriptional modifications, non-sense mediated decay, or protein truncation. In fact, a pan-cancer analysis demonstrated an increased in AS events in adult cancer versus normal tissue (Kahles et al., 2018). More recently, it has been shown that AS targets cancer genes more efficiently than point mutations across a range of different kind of cancers, and

particularly in both pediatric and adult high-grade diffuse glioma (HGG), which are associated to a worse prognosis (Siddaway et al., 2022).
Guanine-rich RNAs have high propensity to fold in G-quadruplex (G4) secondary structures. Emerging evidence has highlighted the involvement of RNA G4s in key cellular processes including translation regulation, 3'-end processing, and mRNA localization (Dumas, L. et al. 2021). In addition, recent bioinformatics analysis and minigene experiments have shown a pronounced enrichment of G4s at weak splice sites and provided evidence for their role as splicing modulators (Georgakopoulos-Soares et al., 2022), corroborating previous works (Huang, H. et al., 2017, Herviou, P., et al. 2020). The fact that RNA G4 folding can be controlled by external factors (*e.g.*, G4 ligands) opens a new way to modulate AS for therapeutic purposes.

Objectives
In this project, we aim to clarify the role of RNA G4s in alternative splicing events in cancer driver genes associated to the oncogenic pathway activation mechanism in HGG, chosen as a cancer model. RNA G4 folding can be affected by the presence of G4 ligands, therefore, we may be able to modulate AS events for therapeutic purposes. We address this goal by making use of RNA structure/function elucidation techniques that couple potent small synthetic molecules with transcriptional approaches to directly and globally map G4 structures located in pre-mRNA sequences and reveal their functional and regulatory roles in mRNA isoform distribution in alternative spliced cancer genes associated to HGG. The final goals of this work are: i) identification of RNA G4-prone sequences in proximity of splicing sites employing bioinformatics tools, ii) in vitro validation of identified-G4 folding by combining biophysical and biochemical probing assays, iii) characterization of well-established and newly designed non-covalent and covalent G4 ligand biophysical activity (RNA G4 binding), and iv) cell viability experiments, RT-qPCR of determined cancer drivers associated to HGG and RNA-seq experiments to identify RNA isoforms produced prior and after ligand treatment in glioma cell lines and clarify the possibility to use G4 at splice sites to reprogram RNA maturation.

Experimental approaches
To identify putative G4 sequences in pre-mRNAs in which alternative splicing has been found deregulated in HGG, we will used bioinformatics algorithms such as G4Hunter and Quadparser and RNA structure software. Bioinformatics studies will be carried out in collaboration with Dr. JL Mergny and V. Brázda. To confirm identified-G4 folding in solution, well-established biophysical methods will be employed (CD spectroscopy, TDS (Thermal Difference Spectra) and IDS (Isothermal difference Spectra)). The latter will be reinforced by biochemical analyses such as SHAPE (Selective 2'-Hydroxyl Acylation analyzed by Primer Extension) probing with semi- quantification analysis. The binding of benchmark and newly designed non-covalent and covalent G4 ligands, previously established by us, to the selected RNA G4s will be subsequently studied by *in vitro* biophysical and biochemical techniques (UV absorbance and Circular Dichroism spectroscopies, fluorescent-based assays developed in house, and gel analysis). Finally, for most promising compounds, the effects produced on AS events will be investigated in HGG cell models (e.g., U-118, U-87 and T98G cell lines) by cell viability experiments, RT-qPCR, and RNA-seq. At last, covalent G4 ligands will be used to identify the exact G4 ligand binding sites on RNA sequences by chemical immunoprecipitation (Chem-IP-seq), a sequencing method developed by us.

International, interdisciplinary & intersectoral aspects of the project

An important part of the project involves a collaboration with the team of Václav Brázda at IBP (Brno, Czech Republic). Specifically, bioinformatics analyses of data originated from RNA-seq experiments, performed in the presence of the most promising G4 ligands in HGG cell models, will be supported by the strong expertise of the research group of Václav lab. This collaboration has already been shown to be successful since two papers have been published (Cantara, A. et al., *Nucleic Acids Res*. **2022**, Brázda, V. et al., *Biomolecules*, **2020**).

The corresponding part of the project will be implemented through mutual, middle term (2–3 months) secondments of PhD students at the two partner institutions (i.e., Institut Curie and IBP). An additional support for this bilateral collaboration is expected through the PHC Barrande Program managed by Campus France (grant application will be submitted in 2024).

Novel G4 ligands discovered within the framework of this project will be protected through patent applications as potential drug candidates for use in cancer treatment of high-grade glioma. Patent protection of novel molecular entities provides potential for creation of a spin-off devoted to pharmaceutical development of new G4 ligands as splicing modulators, with a long-term goal of generation of drug candidates for clinical studies.

This research project crosses several scientific domains, namely bioinformatics (identification of G4 putative sequences transcriptome-wide and RNA-seq data treatment), molecular biophysics (*in vitro* studies of RNA G4 folding and ligand interactions with G4 RNAs), biochemistry (SHAPE probing) and cellular biology (RT-qPCR and RNA-seq studies and evaluation of the therapeutic potential). The required expertise in these domains is ensured by the project consortium involving the PhD supervisor (Dr. D. Verga: in vitro biophysics and biochemistry and in cell studies), a French collaborator (Dr. JL. Mergny: biophysics and bioinformatics) as well as the international partner/mentor (Dr V. Brázda: RNA-seq data analysis by Bioinformatics).

Recent publications

1. Luo, Y.; Živković, M. L.; Wang, J.; Ryneš, J.; Foldynová-Trantírková, S.; Trantírek, L.; **Verga, D.***; Mergny, J.-L.* "A sodium/potassium switch for G4-prone G/C-rich sequences." *Nucleic Acids Res.* **2023**.

2. Esnault, C.; Magat, T.; Zine El Aabidine, A.; Garcia-Oliver, E.; Cucchiarini, A.; Bouchouika, S.; Lleres, D.; Goerke, L.; Luo, Y.; **Verga, D.**; Lacroix, L.; Feil, R.; Spicuglia, S.; Mergny, J.-L.; Andrau, J.-C.* *"G4access identifies G-quadruplexes and their associations with open chromatin and imprinting control regions."* *Nat. Genet.* **2023,** *55*, 1359-1369.

3. Lena, A.; Benassi, A.; Stasi, M.; Saint-Pierre, C.; Freccero, M.; Gasparutto, D.; Bombard, S.; Doria, F.*; **Verga, D.*** "*Photoactivatable V-Shaped Bifunctional Quinone Methide Precursors as a New Class of Selective G-quadruplex Alkylating Agents*". *Chem. Eur. J.* **2022**, *28*, e20220073.

4. Luo, Y.; **Verga, D.***; Mergny, J.-L.*. "*Iso-FRET: An isothermal competition assay to analyze quadruplex formation in vitro*". *Nucleic Acids Res*. **2022**, *50* (16), e93.

5. Masson, T.; Landras Guetta, C.; Laigre, E.; Cucchiarini, A.; Duchambon, P.; Teulade-Fichou, M.P.*; **Verga, D***. "*BrdU immuno-tagged G-quadruplex ligands: a new ligand-guided immunofluorescence approach for tracking G-quadruplexes in cells" Nucleic Acids Res*. **2021**, *49*, 12644–12660.

Expected profile of the candidate

The candidate should have robust background in chemistry, biochemistry and/or pharmaceutical sciences and experience in biophysical and biochemical assays with a strong interest in chemical biology. He/she should be highly motivated to be involved in a multidisciplinary project combining biophysical studies, biochemistry and basic bioinformatics. He/she should have a master degree in chemistry or pharmaceutical sciences with know-how in biochemistry and biophysics. The candidate will have to communicate with collaborators and be comfortable in interacting with different scientists.