COLLEGE DOCTORAL BRETAGNE SCIENCES DE LA VIE ET DE LA SANTE

Year 20 /20

## THESIS TOPIC

| **Subject N° (to be completed by the ED):** | **FUNDING:** ☒ **Requested** ☐ **Acquired** | **Funding origin:** |
|---|---|---|
| Thesis title:<br>**Functional consequences of *SF3B1* mutations in cancer: exploration of splicing modulation as a therapeutic avenue.** | | 3 keywords:<br>**cancer, RNA splicing, *SF3B1*** |
| Unit / team: **UMR1078 / Astre** | | |
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Socio-economic and scientific context (approximately 10 lines):
About 95% of coding genes in humans are subject to alternative splicing, a highly regulated complex mechanism that allows the diversification of the proteome by creating multiple proteins from the same gene. Alterations in alternative splicing are common in cancer, and are often associated with the presence of somatic mutations in genes encoding components or regulators of the splicing machinery. Among these genes, *SF3B1 (splicing factor 3B subunit 1)* is the most frequently mutated in cancer, particularly in myelodysplastic neoplasia (MDS), which is characterized by ineffective myelopoiesis and a risk of transformation into acute myeloid leukemia. In MDS, *SF3B1* mutations are associated with the presence of ring sideroblasts (RS) in the bone marrow, reflecting iron metabolism defects in the erythroid lineage. *SF3B1* mutations are considered as driver mutations, intervening very early in the development of the disease. Research on SF3B1 is expanding internationally, as evidenced by the large number of articles published in the field. Nevertheless, the molecular mechanisms involved are still largely unknown. *SF3B1* is mutated in several cancers for which the therapeutic options remain limited (MDS, pancreatic and breast cancers, uveal melanoma), which underlines the importance of seeking new therapeutic avenues. This research project recently obtained the support of the regional committee of "Ligue contre le cancer".

Working hypothesis and aims (approximately 8 lines):
Cancer-associated *SF3B1* mutations lead to a remodeling of the transcriptome, the functional consequences of which remain to be explored. Hundreds of alternative transcripts are produced: part of them are recognized and degraded by the mRNA quality control machinery, while others would lead to the genesis of new protein isoforms. Among the alternative transcripts produced upon expression of *SF3B1* mutations, we hypothesize that some of them would play a major role in the development of the disease. Therefore their study seems essential to better understand the impact of *SF3B1* mutations in the pathophysiology of MDS, and in the longer term to propose new therapeutic approaches. This thesis project aims to better understand the functional consequences of *SF3B1*, especially on splicing and iron metabolism, and to explore new therapeutic strategies based on the targeted splicing modulation of genes of interest. We propose to develop RNA-based therapies to correct splicing events that would reduce anemia in MDS with *SF3B1* mutations (axis 1), or generate splicing profiles which would sensitize *SF3B1* mutated cells to pharmacological inhibitors or which would induce synthetic lethality (axis 2).

Main milestones of the thesis (approximately 12 lines):
Axis 1- In the continuity of our previous work, we aim to better understand the molecular mechanisms contributing to the defect of iron metabolism and erythropoiesis in MDS-RS, and to those contributing to the physiopathology of MDS. The comparative study of RNAseq and proteomic data generated in the same cellular background (mutated versus wild-type SF3B1) enabled us to identify pathways and genes of interest, whose expression or splicing will be modulated to test their possible involvement in the pathophysiology of MDS. We will use several cell models, as well as primary cells from the bone marrow of MDS patients (sample collection from Brest University Hospital). Axis 2- We aim to exploit the vulnerabilities of mutated-*SF3B1* cells to explore a new therapeutic strategy designed to eliminate the tumor clones. We will use data from the literature and from our lab to establish a list of candidate genes whose alteration could lead to synthetic lethality in combination with *SF3B1* mutations. For both axes, we will use antisense oligonucleotides that bind to canonical or splicing regulatory sequences to modulate splicing at junctions of interest. We will introduce these molecules into cell lines and primary cells (mutated and wild-type SF3B1) to study their effects on phenotypes of interest at the cellular level (Axis 1) or on cell proliferation and cell death, alone or in combination with pharmacological inhibitors (Axis 2). Depending on the results obtained during the first year of the thesis, one of the axes may be further developed.

Scientific and technical skills required by the candidate (2 lines):
Master 2 in Genetics, Molecular Biology, Cancerology or Cellular Biology. Required skills: molecular biology techniques, cell culture and biochemistry. Bioinformatics skills to analyse transcriptomic data will be appreciated.

3 publications from the team related to the topic (last 5 years):
SA. Mian, C. Philippe, E. Maniati, P. Protopapa, T. Bergot, M. Piganeau,T. Nemkov, D. Di Bella, V. Morales, AJ. Finch, A. D'Alessandro, K. Bianchi, J. Wang, P. Gallipoli, S. Kordasti, AS. Kubasch, M. Cross, U. Platzbecker, DH. Wiseman, D. Bonnet, DG. Bernard, JG. Gribbe, K. Rouault-Pierre. Vitamin B5 and Succinyl-CoA improve ineffective erythropoiesis in SF3B1 mutated myelodysplasia. Sci Transl Med. 2023 Mar;15(685):eabn5135. doi: 10.1126/scitranslmed.abn5135. Epub 2023 Mar 1.

Douet-Guilbert N, Soubise B, Bernard DG, Troadec MB. Cytogenetic and Genetic Abnormalities with Diagnostic Value in Myelodysplastic Syndromes (MDS): Focus on the Pre-Messenger RNA Splicing Process. Diagnostics (Basel). 2022 Jul 7;12(7):1658. doi: 10.3390/diagnostics12071658.

Bergot T, Lippert E, Douet-Guilbert N, Corcos L and Bernard DG. Human cancer-associated SF3B1 mutations lead to a splicing modification of its own RNA. Cancers (Basel). 2020 Mar 11;12(3). pii: E652. doi: 10.3390/cancers12030652

National and international collaborations:

Dr P. Stirling (British Columbia Cancer Research Centre, Vancouver, Canada)

Dr K. Rouault-Pierre (Queen Mary University, Londres, RU)